THE
BIOLOGICAL BULLETIN

PUBLISHED BY
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THE BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

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II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips, and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The members of the Corporation shall consist of persons elected by the Board of Trustees.

II. The officers of the Corporation shall consist of a President, Vice President, Director, Treasurer, and Clerk.

III. The Annual Meeting of the members shall be held on the Friday following the second Tuesday in August in each year at the Laboratory in Woods Hole, Massachusetts, at 9:30 A.M., and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years, and shall transact such other business as may properly come before the meeting. Special meetings of the members may be called by the Trustees to be held at such time and place as may be designated.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

VI. Inasmuch as the time and place of the Annual Meeting of members are fixed by these By-laws, no notice of the Annual Meeting need be given. Notice of any special meeting of members, however, shall be given by the Clerk by mailing notice of the time and place and purpose of such meeting, at least fifteen (15) days before such meeting, to each member at his or her address as shown on the records of the Corporation.

VII. The Annual Meeting of the Trustees shall be held promptly after the Annual Meeting of the Corporation at the Laboratory in Woods Hole, Mass. Special meetings of the Trustees shall be called by the President, or by any seven Trustees, to be held at such time and place as may be designated, and the Secretary shall give notice thereof by written or printed notice, mailed to each Trustee at his address as shown on the records of the Corporation, at least one (1) week before the meeting. At such special meeting only matters stated in the notice shall be considered. Seven Trustees of those eligible to vote shall constitute a quorum for the transaction of business at any meeting.

VIII. There shall be three groups of Trustees:

(A) Thirty-two Trustees chosen by the Corporation, divided into four classes, each to serve four years. After having served two consecutive terms of four years each, Trustees are ineligible for re-election until a year has elapsed. In addition, there shall be two groups of Trustees as follows:

(B) Trustees ex officio, who shall be the President and Vice President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer, and the Clerk:

(C) Trustees Emeriti, who shall be elected from *present* or *former* Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next Annual Meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee *Emeritus* for life. The Trustees *ex officio* and *Emeritus* shall have all the rights of the Trustees except that Trustees *Emeritus* shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

IX. The Trustees shall have the control and management of the affairs of the Corporation; they shall elect a President of the Corporation who shall also be Chairman of the Board of Trustees and who shall be elected for a term of five years and shall serve until his successor is selected and qualified; and shall also elect a Vice President of the Corporation who shall also be the Vice Chairman of the Board of Trustees and who shall be elected for a term of five years and shall serve until his successor is selected and qualified; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. The Board of Trustees shall have the power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

X. The Associates of the Marine Biological Laboratory shall be an unincorporated group of persons (including associations and corporations) interested in the Laboratory

and shall be organized and operated under the general supervision and authority of the Trustees.

XI. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

XII. The account of the Treasurer shall be audited annually by a certified public accountant.

XIII. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

IV. REPORT OF THE DIRECTOR

To: The Trustees of the Marine Biological Laboratory

Gentlemen:

I submit herewith the report of the seventy-second session of the Marine Biological Laboratory.

During the past year the Laboratory made significant progress in rehabilitating some of its research space and facilities and also funds were obtained for a new research building and additional housing.

1. Policy

During the past several years there have been about thirty people including investigators and their co-workers engaged in research on a year-round basis at the Laboratory. Investigators have made application through the Laboratory to various granting agencies for support for their various projects. In general, such investigators have been provided with laboratory space and services for a limited tenure, usually no more than five years. In addition, there have been other investigators, either retired or on sabbatical leave, who have availed themselves of the opportunity to work at the Laboratory continuously for one or more years at the discretion of the Executive Committee.

There has been a growing interest among the members of the Corporation, particularly some of those engaged in invertebrate zoological research, in the possibility of developing year-round research programs at the Laboratory in marine systematics and ecology. It is felt by many that such programs will serve to strengthen the summer research programs and will represent the most profitable type of year-round research. The Board of Trustees concurred in a recommendation from the Executive Committee that such a combined systematics-ecology program be operated by the Laboratory, the staff to be selected by the Executive Committee. Efforts are being made to receive support for this program. Also, a grant has been obtained from the Office of Naval Research with which to explore the feasibility of developing a year-round research program in marine microbiology.

The Laboratory will continue to make research space and facilities available to

retired scientists and those on sabbatical leave on the approval of the Executive Committee.

2. Research Training Programs

In 1959 the course in Physiology operated as a research training program under the direction of Dr. William McElroy with support from the National Institutes of Health. The success of this operation strongly supported the desirability of developing the other courses along similar lines. Research training program support has been obtained from the National Institutes of Health for Experimental Embryology starting in 1960 and for Marine Ecology starting in 1961. During this present winter the Old Lecture Hall has been completely remodeled to house the Experimental Embryology Training Program. Also in 1960 Invertebrate Zoology and Marine Botany will start operating as research training programs with support from the National Science Foundation. It is confidently anticipated that these programs will attract staff members of the same high caliber as have the courses in the past. At this writing the trainee applicants far exceed the numbers that can be accommodated in the various programs, which permits the selection of highly qualified individuals.

All the training programs will run for the entire summer season. Each program will operate in the manner best calculated in the minds of its director and his staff to develop background knowledge, technical competence and research interest in the trainees. It is anticipated that these programs will stimulate increased interest in marine biology since it will introduce the beginning investigator to marine research material early in his career and emphasize the unique advantages that such material presents for a great variety of problems.

3. New Laboratory Building

The current progress on the new building assures completion for 1960 summer occupancy barring unforeseen delays in the construction. The schedule is a very tight one so any interruption of the work will be most serious. Mr. Homer P. Smith, General Manager of the Laboratory, merits the highest commendation for the energetic and effective way in which he has promoted the construction of the building. The original planning called for completion of the building for 1961 summer occupancy so that the present schedule represents the gain of a full year.

4. Grants, Contracts and Contributions

The total income from these sources of support amounted to $181,726.00 in 1959. This represents 29.6% of the total income and is made up of the following accounts:

American Cancer Soc.—RC4A (+) Studies in Radiobiology	$ 3,300.00
AEC—1343—Program of Research on the Physiology of Marine Organisms Using Radioisotopes	16,165.00
NIH—4359—Biological Research on the Morphology, Ecology, Physiology, Biochemistry and Biophysics of Marine Organisms	40,000.00
NIH—5143—Training Program in Nerve Muscle Physiology	21,654.00

ONR—1497—Studies in Marine Biology	15,000.00
ONR—09701—Studies on Isolated Nerve Fibers	6,072.00
ONR—09702—Studies in Ecology	3,055.00
NSF—8295—Studies in Marine Biology	40,000.00
AEC–MBL–BM–78–59—Equipment	13,690.00
MBL Associates	4,170.00
Abbott Laboratories	1,000.00
Carter Products	1,000.00
Ciba Pharmaceutical Products, Inc.	1,000.00
Josephine B. Crane Foundation	2,000.00
Eli Lilly & Company	5,000.00
Merck Company Foundation	1,000.00
Schering Foundation, Inc.	1,000.00
Smith, Kline & French Foundation	3,000.00
The Upjohn Company	1,000.00
Wyeth Laboratories, Inc.	1,000.00
Olin Mathieson Chemical Corporation Charitable Trust	500.00
George Frederick Jewett Foundation	1,000.00
Miscellaneous Individual	120.00
	$181,726.00

5. Future Plans

During the summer of 1959 the Executive Committee in several meetings considered the immediate future needs of the Laboratory and recommended that steps be taken to (1) develop plans and obtain funds for a dormitory-dining hall building, (2) construct an additional 25 cottages on the Devil's Lane Property, and (3) prepare an application for funds to cover the detailed planning and construction of a research instruction building.

The concentration of both the research and dormitory buildings on our present campus creates a serious problem of congestion. The ten old wooden residences used as dormitories take up space out of proportion to the numbers they accommodate. Also they do not adapt satisfactorily to dormitory use. The present dining hall, constructed for table service, has been modified to cafeteria service but it leaves much to be desired in fully meeting the needs of the Laboratory. A new dormitory-dining hall facility is urgently needed in a convenient location off of our present limited campus. There were sixty applications in 1959 for the twenty-five new cottages built in the spring in the Devil's Lane Tract. An additional twenty-five cottages are urgently needed particularly since the training programs will attract a larger number of advanced students. A research training building to replace the wooden laboratories will provide modern facilities for the training program staffs and the trainees. Such a building will permit the consolidation under one roof of the variety of services which must be provided the present training programs housed in three wooden buildings. It will also permit certain economies in operation and maintenance.

Respectfully submitted,
PHILIP B. ARMSTRONG,
Director

MEMORIAL

Edmund Newton Harvey

by

Aurin M. Chase

The death of Edmund Newton Harvey from a heart attack on the morning of July 21st at his Penzance Point home was so unexpected as even now hardly to seem possible to his multitude of friends. Indeed, so difficult is it to realize that for a long time many of those who knew him will still expect to hear his voice in the corridors of the M.B.L., or to see him at their doors asking what's new, or reporting, with that characteristic enthusiasm, the most recent discovery in bioluminescence or in any one of a dozen other fields.

It is rare indeed for a man to reach the age ordinarily associated with retirement and still remain so young. Newton Harvey was equally at home with people of all ages, and they with him. He will be greatly missed at Woods Hole and the many other places where he lived, worked and played.

Having graduated from Germantown Academy and then received his Bachelor of Science at the University of Pennsylvania, Harvey went to Columbia for his graduate work and was awarded the Ph.D. in 1911. He started teaching immediately at Princeton where, virtually single-handed, he initiated courses in general physiology and biochemistry, subjects not often included in biology curricula at that time. In 1916 he married Ethel Nicholson Browne, herself a Columbia Ph.D. in zoology. They shared laboratories at Princeton and Woods Hole over the years, except for the period when it was necessary for her to devote full time to raising their two sons, Ned and Dick. The Harveys had a host of friends and greatly enjoyed interesting company. Many will remember, for example, the "Harvey Table" at the M.B.L. Mess as a center of good conversation and congeniality.

At Princeton, Newton Harvey was promoted to full professor in 1919 and, fourteen years later, became Henry Fairfield Osborn Professor of Biology, occupying that chair until his retirement in 1956. During his years of teaching he attracted many graduate students, most of whom based their Ph.D. theses on some aspect of bioluminescence. Nearly all his summers were spent at the M.B.L., where he had been elected a member of the Corporation in 1910, when barely out of college, and became a trustee in 1929. He served as Vice President of the Corporation from 1942 until 1952, and was always most active and influential in the affairs of the Laboratory. He was elected Trustee Emeritus in 1958. Although it was only natural that a man of his interests and energy should become involved in the direction and operation of many organizations—he was, for example, a trustee of the Bermuda Biological Station—the activities and welfare of the Marine Biological Laboratory were always closest to his heart.

With as many interests as he had, it is not surprising that Harvey belonged to some twenty societies covering the fields of physiology, biochemistry and biophysics, as well as less specialized areas of biology. He was a member of The National Academy of Sciences and The American Philosophical Society, and had been vice president and president of The International Society of Cell Biology. In 1953 he was president of The American Society of Zoologists, as he was also of The American Society of Naturalists in 1956. He was an associate editor of several journals, and served as Managing Editor of *The Journal of Cellular and Comparative Physiology* during the first sixteen years of its existence.

Among the formal awards made to him were the John Price Wetherill Medal of The Franklin Institute, in 1934, for his development of the centrifuge-microscope, and the Rumford Medal, by The American Academy of Arts and Sciences, in 1947, for his work

in bioluminescence. He received the Certificate of Merit of the U. S. Armed Forces for his services, both experimental and advisory, during the second world war. More recently, the Johns Hopkins University awarded him an honorary Doctor of Science degree, and Temple University the honorary degree of Doctor of Laws.

Harvey's attitude toward research was always that of the explorer and pioneer. He opened up new regions for others to develop. His tremendous curiosity and drive were not satisfied by the sort of routine experimentation required to wrap up completely all the loose ends of a problem. Without his kind, the discovery of new things would be slow if it occurred at all. So extensively did he explore in the field of bioluminescence, for example, that it is difficult to do any experiment involving a luminous organism without finding, sooner or later, that Harvey had had the same idea and tested it—at least in a preliminary way—years before.

A person of such consuming curiosity could never be satisfied in a single line of research. His 250 or so published papers include such topics as cell permeability and oxidations, physical measurements at cell surfaces, brain potentials, effects of supersonic waves and of centrifugal forces on cells and—during the war—decompression sickness and wound ballistics. He loved instrumentation and was always eager to apply new apparatus and techniques to biological problems. His vast knowledge of organisms was most useful in this connection. But his greatest interest, and the one to which he returned again and again, and which was occupying him at the time of his death, was bioluminescence. It is for this that he is best known and the acknowledged authority throughout the world. He wrote four books on the subject, and was working on a fifth.

One who did not know Newton Harvey might get the mistaken impression from a review of his many scientific accomplishments that his time was spent entirely in the laboratory and the study. Nothing could be farther from the truth! He enjoyed life to the full! He was an excellent tennis player in his time and an experienced mountain climber, went on numerous scientific expeditions, and at one time was even reported to have been eaten by cannibals! At Woods Hole he always found time for swimming and sailing, and the Harveys' sloop, the "Rip Tide," carried many a happy party across Vineyard Sound to Edgartown, or down the islands to Tarpaulin Cove or Cuttyhunk.

He loved to surprise his friends in unusual and pleasant ways, such as pulling off the road into a secluded grove, opening the luggage compartment of his big Buick, and serving cocktails from the portable bar which nobody had suspected was there. Then would follow dinner at some nearby restaurant which he had previously tested and found to be good.

Yet in the midst of a social gathering his scientific interest might suddenly come to the fore. As when, once, he tossed a corked bottle to the ceiling so that all could observe that the large air bubble trapped inside did not rise in the bottle while it was in "free fall."

All those who have come in contact with Edmund Newton Harvey, his friends, colleagues, graduate students, and those fortunate undergraduates who did their senior theses under his direction, have absorbed some of his boundless enthusiasm and spirit. Certainly he will be sorely missed. But surely he would not have preferred to have gone otherwise than as he did; in full possession of all his faculties; vigorous, joyful and active until the last.

MEMORIAL

George H. A. Clowes

by

Philip B. Armstrong

Rarely do we encounter in one man such a variety of talents and interests as characterized George Henry Alexander Clowes. With unbounded energy, he used these talents effectively, following up his interests with persistent determination. He was a

scientist and investigator who sparked many practical applications of basic research. He was a man of business, a civic leader, and patron of the fine arts and of music.

Dr. Clowes was born in Ipswich, England, in 1877, in a period of developing scientific interest. Through environment and natural bent, he early developed an interest in science. His family was in the business of producing intermediates for the brewing industry.

He attended the Royal College of Science in London from 1893–1896 and completed his studies for a Ph.D. degree in chemistry in 1899 at the University of Göttingen. His thesis was concerned with the methyl derivatives of sugar. After additional work in some of the other leading chemical laboratories in France and Germany, he came to this country in 1901 as chemist at the Institute for the Study of Malignant Diseases at Buffalo.

For the following period of fifteen years, Dr. Clowes published extensively, using a variety of approaches to the problem of cell division and growth, particularly as it applied to cancer. An important contribution concerned the evidence for immunity against cancer in mice after spontaneous remissions. Other papers dealt with the mechanism of the action of mustard gas, enzyme action in fermentation, ion antagonisms, and the action of anesthetics in biological and physical systems. His outstanding scientific contributions were on the effects of calcium or sodium at oil-water interfaces. Surface phenomena remained a life-long interest resulting in many practical developments.

Dr. Clowes left Buffalo in 1918 to serve in the Chemical Warfare Service where he continued his work on mustard gas, particularly its mechanism of action. In order to study its action under most favorable experimental conditions, he spent the summer of 1918 at the Marine Biological Laboratory, working with Robert Chambers and Ralph Lillie. Although Dr. Clowes had a highly practical turn of mind, he had a deep appreciation of the potential significance of basic research. The scientific intellectual atmosphere of the Marine Biological Laboratory was so attractive to Dr. Clowes that he spent most of the following 40 summers at Woods Hole. At the Laboratory he found what he considered ideal biological materials for many of the basic problems in which he was interested.

After the war he joined the research staff of Eli Lilly and Company and became Director of Research in 1921. Here he served very effectively in the development of the commercial production of several products, starting with insulin. His earlier interest in salt antagonisms and the effects of pH on proteins served him in good stead in the precipitation and purification of insulin. He also played a prominent role in the development of liver extract, protamine insulin and penicillin. From the Marine Biological Laboratory with collaborative workers Homer Smith, Maurice Krahl, Anna Keltch and others, Dr. Clowes published a series of papers on the possible control of mitosis by chemical agents.

In 1918 he was elected a member of the Corporation of the Marine Biological Laboratory, served three terms as a trustee, and was elected a Trustee Emeritus in 1948. He was active in seeking support for the Laboratory as well as contributing generously himself. In Indianapolis he played an active role in the development of the Indianapolis Symphony Orchestra and the John Huron Art Institute.

Dr. Clowes enjoyed a felicitous marriage. He is survived by his wife, Mrs. Edith Whitehill Clowes, who shared with him many of his civic and philanthropic interests. Also surviving him are two sons, Dr. G. H. A. Clowes, Jr. and Allen W. Clowes. Dr. Clowes died in Woods Hole on August 25, 1958. He will be remembered by his Woods Hole friends for his outstanding generosity and kindness and his active participation in the scientific and physical development of the Marine Biological Laboratory. He had a keen interest in the work of others, participated actively in the scientific discussion at the Laboratory, and was always ready to give help in the development of science.

MEMORIAL

Eugene Floyd Du Bois

by

Paul Reznikoff

Dr. Eugene Floyd Du Bois died on February 12, 1959, at his home at the age of 76. His death is not only a great loss to medicine and physiology but to his many students, house officers and colleagues who were associated with him at Cornell University Medical College, Bellevue Hospital, the New York Hospital and the Marine Biological Laboratory, it is a personal tragedy.

Doctor Du Bois was born on June 4, 1882 in West New Brighton, Staten Island, New York, the son of Eugene and Anna Brooks Du Bois. He attended the Staten Island Academy and Milton Academy in Massachusetts and received his A.B. degree from Harvard in 1903 and his M.D. degree from Columbia in 1906. In 1948 he was awarded an honorary Doctor of Science degree by Rochester University.

After interning at the Presbyterian Hospital (1907–08) and acting as assistant pathologist (1909) at this institution he decided to secure a training in bacteriology. But just before he was about to leave for France he changed his plans at the suggestion of Dr. John Howland and he went to Germany to study metabolism. Dr. Graham Lusk visited the laboratory in Berlin and met Dr. Du Bois there. This was the beginning of a lifelong association of these two pioneers in the field of metabolism. When Dr. Du Bois returned to the United States he soon became the Medical Director of the Russell Sage Institute of Pathology, of which Dr. Lusk was the Scientific Director. Thus Dr. Du Bois became a scientific descendant of Lavoisier. Under Du Bois' guidance the Russell Sage Institute has had world-wide influence in advancing scientific knowledge in the field of metabolism and from his "Calorimeter Room" there have gone forth many of our most important scientists and medical educators.

Doctor Du Bois was Director of the Second (Cornell) Medical Division of Bellevue Hospital from 1919 to 1932, Professor of Medicine at the Cornell University Medical College from 1930 to 1941, Physician-in-Chief at the New York Hospital from 1932 to 1941, and Professor of Physiology at Cornell from 1941 to 1950 when he became Emeritus Professor.

Some of Dr. Du Bois' most important contributions were concerned with his work for the United States Navy. Because of his modesty few of his associates knew that he was an outstanding authority in the fields of submarine warfare and aviation medicine. For heroism in the conduct of hazardous experiments during World War I he received the Navy Cross. During the second World War he was recognized by Commendation and Ribbon Bar. He retired from the Navy with the rank of Captain and continued to work with the military service until the very day of his death.

He belonged to many societies—the National Academy of Sciences, the Philosophical Society, the American Physiological Society, the Aero Medical Association, the Society for Experimental Biology and Medicine, and was president of the American Society for Clinical Investigation, of the Association of American Physicians, of the Harvey Society and of the Institute of Nutrition.

He became a member of the Corporation of the Marine Biological Laboratory in 1929 and was elected a Trustee in 1942. He was re-elected in 1944 for an additional full term to 1948. In 1952 he was elected Trustee Emeritus which position he held until his death. He was as conscientious in his duties to the Laboratory as he was in all undertakings and even during the later years of his incapacity he attended the meetings of the Trustees despite his physical limitations. He planned to be present at the Annual Winter Meeting which took place just one day after his death.

Many honors came to Dr. Du Bois, such as the Kober Medal of the Association of American Physicians in 1947 and the Academy Medal of the New York Academy of Medicine in 1956. He was to receive the John Phillips Memorial Award of the American College of Physicians in April.

Doctor Du Bois' accomplishments and honors are of minor importance compared to the influence he has had upon his students and associates by virtue of his personality and character. As his life-long friend and successor as Professor of Medicine, Dr. David P. Barr, has said, "This extraordinary influence has been attributable only in part to his mastery of experimental procedure and the intrinsic value of his scientific contributions. Its essence derives from his own character and personality. Inspiration has come to others from his abiding faith in principles of scientific and personal conduct, from his integrity and tolerance, and from his sympathetic understanding of the problems of those about him. His character has influenced behavior of his colleagues. It has also influenced innumerable students who have learned from him lessons of critical evaluation, clear expression, unvarying courtesy, and true humility."

Doctor Du Bois was a *gentleman, gentle* in all his dealings with his fellow men and with suffering, and a *man* in his uncompromising attitude toward injustice and dishonesty.

A colleague once asked him why he did not delegate some of his difficult and unpleasant problems to his subordinates. His reply was that such tasks were the duties of the chief.

The principles which guided him in educating the medical students were described in an article entitled "The Clinical Clerkship in Medicine," published in the *Journal of the American Medical Association*, August 21, 1926, and were these: "The purpose of instruction is to teach the students to teach themselves; the manner of instruction is by example and work; the spirit of instruction is sympathy for and faith in the students."

All his friends join his widow, his three children and his nine grandchildren in being proud of their association with a great and good man. His life may be summarized by the citation on the Academy Medal: "Eugene Floyd Du Bois, physiologist, physician, educator, patriot. His life and work have brought honor to the profession of medicine" and to science.

MEMORIAL

Jacques Loeb

by

W. J. V. Osterhout

This year marks the 100th anniversary of the birth of Jacques Loeb, who contributed so much to the study of marine biology, and it seems appropriate that in a marine biological station where he worked, we should recall his activities.

Thirty-five years have passed since his death and yet our memory of him is still fresh and vivid.

He was above all an idealist. Protected by his devoted wife who knew how to help him, he lived in a world of ideals. Their inspiration dominated his life and set him apart from others. Yet he had also a tender heart, and his sympathy was always with those who were in need of help.

His outstanding feature was his creative imagination, implying prophetic vision, the intuitive, and emotional urge of ideas.

Fortunately his poetic imagination was associated with a keen critical sense. He would test his conceptions over and over again and repeat his experiments very carefully. He published only a small part of his experimental work. It is remarkable that his observations remain valid without fundamental modifications.

The questions he put to nature were never dull and the answers he received were always interesting and at times startlingly so.

He was not content to pursue a special part of a problem without considering its relation to all the rest. To achieve this, it was necessary both to simplify and to generalize, and these powers he possessed to an extraordinary degree.

Courage played a great part in his success. He did not select problems because they were easy but because of their importance. His courage sprang largely from his faith in the mechanistic conception to which he consecrated his life.

He had a truly lovable and sympathetic personality that drew men irresistibly to him. His teaching was inspiring and unforgettable, so that it was not strange that young men gladly followed him.

One felt instinctively that he cared only for truth and that in its quest he would spare no labor and sacrifice.

The breadth of his knowledge made it natural for him to utilize in his work recent advances in other fields of science. Thus he took the ideas of tropism and of heteromorphosis from botany. He applied to biology theories of dissociation and osmotic pressure which resulted in the discovery of artificial parthenogenesis and antagonistic salt action. To the very end of his life he kept in touch with recent progress in physics and chemistry which he applied to his own studies.

Death came while he was actively engaged in what he regarded as the most fundamental investigation of his life. In the midst of this research on proteins he was stricken down.

Here we may pause to ask ourselves, how are we to remember him? He was an idealist, sympathizing with all suffering, consecrating his gifts to humanity; a scientist with an artist's soul, emotional, intuitive, creative; a thinker, strangely original, born to blaze fresh trails and teach new doctrines; a dreamer, regarding the world of life with poetic insight and seeking with creative imagination rarely equalled to sweep aside its mystery and set free the mind of men. His visions, that have made others see visions, cannot but continue to shed inspiration; and in shaping the soul of the future he may serve humanity more than he dared to dream.

MEMORIAL

Frank M. MacNaught

by

Charles Packard

Long and faithful was the service rendered to this Laboratory by Frank M. MacNaught who died in June of this year at the age of 83. Coming to Woods Hole as an accountant in 1913 when the Laboratory began its rapid growth, he was soon made Registrar, and then, in 1916, the Business Manager, a position which he held for 34 years. For much of this time his only assistant was Miss Polly Crowell. Among his many responsibilities was the task of assigning laboratory rooms and tables, and the much more difficult work of apportioning space in the Apartment House and dormitories. Only those who worked closely with him can appreciate the care which he exercised in selecting places best adapted to the needs of each applicant. In addition to these duties, he was responsible for the Mess and its many employees.

From the first he devoted himself to these various tasks, discharging them with great efficiency. Always in the Office, even on Sundays and holidays, he was quick to help newcomers unfamiliar with the operation of the Laboratory. His memory was extraordinary. He could at once call by name investigators and students returning after an

absence of many seasons, even recalling the year of their last attendance and the rooms they occupied. His friendliness endeared him to all. He once remarked that if he should take a trip across the country he could spend each night at the home of investigators or students who had especially asked him to visit them. In his relations with all he worked with he showed patience, good judgment, and great tact. Many times he relieved a tense situation with an apt, humorous remark.

He was active in Town affairs, serving on the Finance and other committees, and in the village, as Treasurer and Trustee of the Woods Hole Public Library, and Clerk of the Coonamesset Ranch.

The Laboratory has lost an exceptional man, a devoted friend whose outstanding services and genial personality will long be remembered.

MEMORIAL TO CHARLES R. CRANE ON HIS HUNDREDTH ANNIVERSARY

by

Lawrason Riggs

On the eighth day of August 100 years ago was born the greatest benefactor and friend of the Marine Biological Laboratory, Charles R. Crane.

Mr. Crane was most interested in education. I think this was because he had no formal education beyond grade school. His father, the founder of the Crane Company, did not believe in colleges, in fact he wrote a book against college education, enlisting in the writing of the book two employees of the Crane Company, both of whom turned out to be college graduates.

He went to work at an early age for his father. In 1878 he happened to be in New York. He wandered down to Front Street where in those days the bowsprits of sailing vessels projected over the street. Young Crane went aboard one of these ships and on telegraphic consent from his father arranged to sail on her to Java as the sole passenger. The only additions he made to his baggage were a set of Herbert Spencer and 12 dozen bottles of Guiness Stout. On the voyage he read the Spencer, drank a bottle of stout every day and learned navigation, and on his 21st birthday he furled the main royal in a gale off the Cape of Good Hope. Toward the end of the voyage the first mate died and Mr. Crane was offered his position. He did not accept as he wanted to see as much of Asia as possible. This was fortunate as on the ship's return voyage the captain and most of the crew died, undoubtedly of beri-beri. Later his doctor informed him that his health had been preserved by those 144 bottles of Guiness Stout.

So began his informal education, which he pursued with unrelenting vigor so that in time he became one of the best informed Americans about the Moslem world, Russia and the Far East. During the Peace Conference after World War I he was appointed on a Commission with President King of Oberlin to investigate and make recommendations on the future of Syria. This mission further deepened and extended his contacts with the Near East. Subsequently he was for a number of years American Ambassador to China.

He had an extraordinary interest in exotic places and a real flair for people. He was as much at home in Paris, St. Petersburg, Cairo, Damascus, Constantinople, Samarkand and Pekin as he was in Chicago, and he numbered among his friends, presidents, especially President Wilson, cabinet members, educators, judges, Moslem leaders, including the King of Hejaz and Sherif of Mecca and his son Feisal, later King of Iraq.

He made some 32 visits to Russia, penetrated the most remote parts of Asia, includ-

ing Bokhara and the Transoxnas, and went with one servant on horseback through Albania after being deserted by the Turkish bodyguard supplied by the Sultan.

The M.B.L. was not the only beneficiary of his interest in education and research. He was interested in the Near East colleges, especially the American College for Girls on the Bosphorus and the Sofia-American Schools in Bulgaria and also a school in Albania and in many universities in this country. The main purpose of his Foundation, The Friendship Fund, was to assist individuals to get an education and the purpose of the Institute of Current World Affairs, which he also founded, was to train specialists in critical areas under conditions that would develop their talents and personality.

His first important contact with the Marine Biological Laboratory was his joining in an offer of assistance with several other persons through President Harper of Chicago University. This happened in 1901.

When this offer and that of the Carnegie Institution were finally rejected by the Laboratory because the trustees and members did not wish to allow the Laboratory to lose its independence, Mr. Crane became more interested through his brother-in-law, Dr. Frank R. Lillie, and before long was contributing about $20,000 a year towards its expenses.

He had a large part in purchasing real property for the Laboratory and in 1913 provided the first brick building, the so-called Crane Building. He had a very important part in the expansion and endowment of the Laboratory between the years 1919 and 1925. He not only capitalized his annual contribution of $20,000 by a gift of $405,000 to endowment, but he guaranteed to pay any cost of the new Rockefeller Building in excess of $500,000. This guarantee cost him $221,000. He had a large part in interesting Mr. John D. Rockefeller, Jr. and the Rockefeller Foundation, as is clear from Mr. Rockefeller's letter found at page 75 of Dr. Lillie's book.

While his gifts were important and always timely, his appreciation of the spirit of the Laboratory and of its democratic and self-governing organization—a group of scientists running their own affairs—was almost more important. He acted as President from 1904 to 1925.

That the Laboratory came through its early and difficult years and survived to become the great institution that it now is is, I feel, largely due to his help and encouragement.

ZOOLOGY

I. Consultants

F. A. Brown, Jr., Morrison Professor of Zoology, Northwestern University
Libbie H. Hyman, American Museum of Natural History
A. C. Redfield, Woods Hole Oceanographic Institution

II. Instructors

Grover C. Stephens, Assistant Professor of Zoology, University of Minnesota; in charge of course.
John B. Buck, Senior Biologist, National Institutes of Health
Ralph I. Smith, Associate Professor of Zoology, University of California, Berkeley
Bernard L. Strehler, Chief, Cellular and Comparative Physiology, Division of Gerontology, National Institutes of Health
Paul P. Weinstein, Laboratory of Tropical Diseases, National Institutes of Health
Richard C. Sanborn, Professor of Zoology, Department of Biological Sciences, Purdue University

MORRIS ROCKSTEIN, Associate Professor of Physiology, New York University College of Medicine
MILTON FINGERMAN, Assistant Professor of Zoology, Tulane University

III. LABORATORY ASSISTANTS

ROBERT ASHMAN, Wabash College
DONALD HALL, University of Michigan

EMBRYOLOGY

I. INSTRUCTORS

MAC V. EDDS, JR., Professor of Biology, Brown University; in charge of course
PHILIP GRANT, Assistant Professor of Pathobiology, Johns Hopkins University
JOHN W. SAUNDERS, JR., Professor of Zoology, Marquette University
NELSON T. SPRATT, JR., Professor of Zoology, University of Minnesota
MAURICE SUSSMAN, Associate Professor of Biological Sciences, Brandeis University
LIONEL REBHUN, Assistant Professor of Biology, Princeton University

II. LABORATORY ASSISTANTS

CHANDLER M. FULTON, Rockefeller Institute for Medical Research
DAVID S. LOVE, University of Colorado

PHYSIOLOGY

I. CONSULTANTS

MERKEL H. JACOBS, Professor of Physiology, University of Pennsylvania
ARTHUR K. PARPART, Professor of Biology, Princeton University
ALBERT SZENT-GYÖRGYI, Director, Institute for Muscle Research, Marine Biological Laboratory

II. INSTRUCTORS

W. D. MCELROY, Professor of Biology, Johns Hopkins University; in charge of course
FRANCIS D. CARLSON, Associate Professor of Biophysics, Johns Hopkins University
BERNARD D. DAVIS, Professor of Bacteriology, Harvard Medical School
DONALD GRIFFIN, Professor of Zoology, Harvard University
HOWARD SCHACHMAN, Virus Laboratory, University of California
TIMOTHY GOLDSMITH, Fellow, Harvard University
ROBERT LOFTFIELD, Massachusetts General Hospital

III. LABORATORY ASSISTANT

LOUIS OTERO, University of Puerto Rico, Rio Piedras

BOTANY

I. CONSULTANT

WM. RANDOLPH TAYLOR, Professor of Botany, University of Michigan

II. Instructors

Richard C. Starr, Associate Professor of Botany, Indiana University; in charge of course
John M. Kingsbury, Assistant Professor of Botany, Cornell University
Walter R. Herndon, Assistant Professor of Biology, University of Alabama

III. Collector

G. Benjamin Bouck, Columbia University

IV. Laboratory Assistants

Larry Hoffman, University of Texas
Robert W. Korn, Indiana University

ECOLOGY

I. Consultants

Paul Galtsoff, U. S. Fish and Wildlife Service, Woods Hole
Alfred C. Redfield, Woods Hole Oceanographic Institution
Bostwick H. Ketchum, Woods Hole Oceanographic Institution
Edwin T. Moul, Rutgers University
Charles E. Jenner, University of North Carolina
Howard L. Sanders, Woods Hole Oceanographic Institution

II. Instructors

Eugene P. Odum, Alumni Foundation Professor of Zoology, University of Georgia; in charge of course
Howard T. Odum, University of Texas
Harold J. Humm, Associate Professor of Botany, Duke University
John H. Ryther, Marine Biologist, Woods Hole Oceanographic Institution

III. Laboratory Assistant

Richard B. Williams, Harvard University

1. The Laboratory Staff, 1959

Homer P. Smith, General Manager

Mrs. Deborah Lawrence Harlow, Librarian
Carl O. Schweidenback, Manager of the Supply Department
Irvine L. Broadbent, Office Manager
Robert Kahler, Superintendent, Buildings and Grounds
Robert B. Mills, Manager, Department of Research Service

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Mrs. Lila S. Myers
Mrs. Vivien R. Brown
Mrs. Virginia M. Morehouse
Mrs. Loretta J. McCartney
Mrs. Marion C. Chase
Mrs. Vivian I. Manson
Mrs. Shirley A. Elder

LIBRARY

Mrs. M. Verna Hanks
Mrs. Gwendolyn S. Blomberg
Mrs. Naomi Botelho
Albert K. Neal

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Eldon P. Allen
Arthur D. Callahan
Robert Gunning
Walter J. Jaskun
Donald B. Lehy
Ralph H. Lewis
Russell F. Lewis
Alan G. Lunn
Alton J. Pierce
Robert H. Walker, Jr.
James S. Thayer

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Gail M. Cavanaugh
John P. Harlow
Seaver R. Harlow
Mrs. Arlene Brown

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Donald P. Burnham
Milton B. Gray
Geoffrey J. Lehy
Robert O. Lehy
Mrs. Mildred H. Mixson
Robert M. Perry
Bruno F. Trapasso
John J. Valois
Jared L. Vincent
Hallett S. Wagstaff

2. Investigators, Lalor and Lillie Fellows, and Students

Independent Investigators, 1959

Adelman, William J., Assistant Professor of Physiology, University of Buffalo
Allen, Robert D., Assistant Professor of Biology, Princeton University
Amberson, William R., Investigator, Marine Biological Laboratory
Armstrong, Philip B., Professor and Chairman of Anatomy, State University of New York, College of Medicine at Syracuse
Arnold, William, Principal Biologist, Oak Ridge National Laboratory
Baltus, Elyane, Chargé de Course, University of Brussels, Belgium
Bang, Frederik B., Professor of Pathobiology, Johns Hopkins University School of Hygiene
Barth, L. G., Professor of Zoology, Columbia University
Baylor, Martha B., Independent Investigator, Marine Biological Laboratory
Bell, Eugene, Assistant Professor of Biology, Massachusetts Institute of Technology
Benesch, Reinhold, Investigator, Marine Biological Laboratory
Benigna, Sister Maria, Professor of Biology, Saint Joseph College
Bennett, Michael, Research Associate, Columbia University, College of Physicians and Surgeons
Bennett, Miriam F., Assistant Professor of Biology, Sweet Briar College
Benzer, Seymour, Professor of Biophysics, Purdue University
Bergmann, Felix, Research Fellow, College of Physicians and Surgeons, Columbia University
Berman, Mones, Physicist, National Institutes of Health
Bernard, George R., Assistant Professor of Biology, University of Notre Dame
Bernstein, Maurice H., Assistant Professor of Anatomy, Wayne State University
Bishop, David W., Staff Member, Carnegie Institution of Washington
Bosler, Robert, Instructor of Physiological Optics, Johns Hopkins Hospital
Brett, William J., Associate Professor of Biology, Indiana State Teachers College
Brown, Frank A., Jr., Morrison Professor of Biology, Northwestern University

Bryant, Shirley H., Assistant Professor of Pharmacology, University of Cincinnati
Buck, John B., Physiologist, National Institutes of Health, Laboratory of Physical Biology
Burbanck, W. D., Professor of Biology, Emory University
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Cascarano, Joseph, Instructor of Pathology, New York University College of Medicine
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Child, Frank M., Instructor in Zoology, University of Chicago
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Cole, Kenneth S., Chief, Laboratory of Biophysics, National Institutes of Health
Colwin, Arthur L., Professor of Biology, Queens College
Colwin, Laura H., Lecturer, Queens College
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Fingerman, Milton, Assistant Professor of Zoology, Newcomb College of Tulane University
Fishman, Louis, Research Associate, New York University College of Dentistry
Forrest, Hugh S., Associate Professor of Zoology, University of Texas
Frank, Ulrich, Extraordinary Professor, Technical University, Darmstadt, Germany
Friz, Carl T., Public Health Fellow, University of Minnesota
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Fuortes, M. G. F., Physiologist, National Institutes of Health
Furshpan, Edwin, Instructor in Ophthalmic Physiology, Johns Hopkins University
Furukawa, Taro, Instructor in Ophthalmic Physiology, Johns Hopkins University
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MARSHALL, JEAN M., Assistant Professor of Physiology, Johns Hopkins University School of Medicine
MARSLAND, DOUGLAS, Professor of Biology, New York University, Washington Square College
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METZ, CHARLES B., Professor, Florida State University

MIDDLEBROOK, W. ROBERT, Research Fellow, Institute for Muscle Research, Marine Biological Laboratory
MONIER, ROGER, Post-doctoral Associate, University of Paris
MOORE, JOHN W., Associate Chief, Laboratory of Biophysics, National Institutes of Health
NACE, PAUL FOLEY, Associate Professor of Biology, McMaster University
NELSON, LEONARD, Assistant Professor, University of Chicago
NEWTON, JACK W., Research Associate, Brandeis University
ODUM, EUGENE P., Professor of Zoology, University of Georgia
OSTERHOUT, W. J. V., Member *Emeritus*, Rockefeller Institute for Medical Research
PALINCSAR, EDWARD E., Instructor of Biology, Loyola University
PAPACONSTANTINOU, JOHN, Post-doctoral Research Fellow, Carnegie Institution of Washington
PARPART, ARTHUR K., Chairman, Department of Biology, Princeton University
PATERSON, MABEL C., Assistant Professor of Zoology, Vassar College
PERLMANN, GERTRUDE E., Associate Professor, Rockefeller Institute for Medical Research
PROSSER, C. LADD, Professor of Physiology, University of Illinois
RANZI, SILVIO, Full Professor, Istituto di Zoologia, Milano, Italy
RAPPORT, MAURICE M., Professor of Biochemistry, Albert Einstein College of Medicine
RASMUSSEN, HOWARD, Graduate Fellow, Rockefeller Institute for Medical Research
READ, CLARK P., Associate Professor of Parasitology, Johns Hopkins University
REBHUN, LIONEL I., Assistant Professor of Biology, Princeton University
RIESER, PETER, Research Associate, University of Pennsylvania
ROCKSTEIN, MORRIS, Associate Professor of Physiology, New York University College of Medicine
ROSE, S. MERYL, Professor of Zoology, University of Illinois
ROSENBERG, EVELYN E., Associate Professor of Pathology, New York University-Bellevue Medical Center
ROSLANSKY, JOHN D., Research Associate, Princeton University
ROTH, JAY S., Associate Professor of Biochemistry, Hahnemann Medical College
ROTHMAN, ALVIN H., Research Fellow, Johns Hopkins University School of Hygiene
RUDOMIN, P., Research Fellow, College of Physicians and Surgeons, Columbia University
RUSHTON, W. A. H., Reader in Physiology, Trinity College, Cambridge, England
RUSTAD, RONALD C., Instructor in Physiology, Florida State University
RYTHER, JOHN H., Staff, Woods Hole Oceanographic Institution
SANBORN, RICHARD C., Professor of Zoology, Purdue University
SANDEEN, MURIEL I., Assistant Professor of Zoology, Duke University
SANDERS, HOWARD L., Research Associate, Woods Hole Oceanographic Institution
SAUNDERS, JOHN W., Professor of Zoology, Chairman Department of Biology, Marquette University
SCHACHMAN, HOWARD K., Associate Professor of Biochemistry, University of California, Berkeley
SCHUH, REV. JOSEPH E., Associate Professor of Biology and Chairman of Department, Saint Peter's College
SCOTT, SISTER FLORENCE MARIE, Professor of Biology, Seton Hill College
SCOTT, GEORGE T., Professor and Chairman, Department of Biology, Oberlin College
SELIGER, HOWARD H., Guggenheim Fellow, Johns Hopkins University
SKOGLUND, CARL RUDOLF, Associate Professor, Karolinska Institutet, Stockholm, Sweden
SMITH, PAUL FERRIS, Electronics Engineer, Rockefeller Institute for Medical Research
SMITH, RALPH I., Associate Professor of Zoology, University of California, Berkeley
SPECTOR, ABRAHAM, Instructor, Harvard Medical School
SPEIDEL, CARL C., Professor and Chairman of Anatomy, University of Virginia
SPIEGEL, MELVIN, Assistant Professor of Biology, Colby College
SPRATT, NELSON T., Chairman, Department of Zoology, University of Minnesota
SPYROPOULOS, CONSTANTINE S., Neurophysiologist, National Institutes of Health
STARR, RICHARD C., Associate Professor of Botany, Indiana University
STEELE, RICHARD, Associate Professor of Biochemistry, Tulane University
STEINBACH, H. BURR, Professor and Chairman, Department of Zoology, University of Chicago
STEINHARDT, JACINTO, Director, Operations Evaluation Group, Massachusetts Institute of Technology

STEPHENS, GROVER C., Associate Professor of Zoology, University of Minnesota
STETTEN, DEWITT, Associate Director in Charge of Research, National Institutes of Health
STETTEN, MARJORIE R., Biochemist, National Institutes of Health
STEVENS, CHARLES F., Medical Student, Yale University School of Medicine
STONE, WILLIAM, Director of Ophthalmic Plastics Laboratory, Massachusetts Eye and Ear Infirmary
STREHLER, BERNARD L., Chief, Comparative Physiology Section, National Institutes of Health
STRITTMATTER, PHILIPP, Assistant Professor of Biochemistry, Washington University
STUNKARD, HORACE W., Research Scientist, U. S. Fish and Wildlife Service
SUDAK, FREDERICK N., Instructor in Physiology, Albert Einstein College of Medicine
SUSSMAN, MAURICE, Associate Professor, Brandeis University
SZENT-GYÖRGYI, ALBERT, Director, Institute for Muscle Research, Marine Biological Laboratory
SZENT-GYÖRGYI, ANDREW, Investigator, Institute for Muscle Research, Marine Biological Laboratory
TASAKI, ICHIJI, Chief, Special Senses Section, National Institutes of Health
TAYLOR, ROBERT E., Neurophysiologist, National Institutes of Health
TAYLOR, WM. RANDOLPH, Professor of Botany, University of Michigan
TEORELL, TORSTEN, Professor of Physiology, Uppsala University, Sweden
TORCH, REUBEN, Assistant Professor of Zoology, University of Vermont
TROLL, WALTER, Assistant Professor of Industrial Medicine, New York University-Bellevue Medical Center
TSUBOI, KENNETH K., Assistant Professor of Biochemistry, Cornell University Medical College
TWEEDELL, KENYON S., Assistant Professor of Biology, University of Notre Dame
DEVILLAFRANCA, GEORGE W., Assistant Professor of Zoology, Smith College
VILLEE, CLAUDE A., Associate Professor of Biological Chemistry, Harvard University
VINCENT, WALTER S., Assistant Professor of Anatomy, Upstate Medical Center, State University of New York
WAINIO, WALTER W., Associate Professor of Biochemistry, Rutgers University
WATANABE, AKIRA, Research Fellow, College of Physicians and Surgeons, Columbia University
WEBB, H. MARGUERITE, Assistant Professor of Biology, Goucher College
WEINSTEIN, PAUL P., Senior Scientist, National Institutes of Health
WEISS, LEON P., Assistant Professor of Anatomy, Harvard Medical School
WELLS, G. P., Professor of Zoology, University College, London, England
WERMAN, ROBERT, Research Associate, College of Physicians and Surgeons, Columbia University
WHITING, ANNA R., Guest Investigator, University of Pennsylvania
WICHTERMAN, RALPH, Professor of Biology, Temple University
WIERCINSKI, FLOYD J., Research Associate, University of Pennsylvania
WILLEY, C. H., Professor of Biology and Chairman of Department, New York University
WILSON, WALTER L., Assistant Professor of Physiology, University of Vermont College of Medicine
WITTENBERG, JONATHAN B., Assistant Professor of Physiology, Albert Einstein College of Medicine
WRIGHT, PAUL A., Associate Professor of Zoology, University of New Hampshire
ZWEIFACH, B. W., Professor of Pathology, New York University-Bellevue Medical Center
ZWILLING, EDGAR, Associate Professor, University of Connecticut

Lalor Fellows, 1959

BERNSTEIN, M. H., Wayne State University
CROSTI, NICOLETTA, Bryn Mawr College
ECHALIER, G. P. R., Harvard College
GONSE, P. H., University of Pennsylvania
LEONE, V., Istituto di Zoologia, Milano, Italy
MARSH, J. B., University of Pennsylvania
NELSON, LEONARD, University of Chicago
PALINCSAR, E. E., Loyola University
RANZI, SILVIO, Istituto di Zoologia, Milano, Italy
ROSLANSKY, J. D., Princeton University

RUSTAD, R. C., Florida State University
SKOGLUND, CARL, Karolinska Institutet, Stockholm, Sweden
STRITTMATTER, P., Washington University

Lillie Fellow

RANZI, SILVIO, Istituto di Zoologia, Milano, Italy

Grass Fellows

LIPICKY, RAYMOND, University of Cincinnati
STEVENS, CHARLES, Yale University, School of Medicine

Beginning Investigators, 1959

BENSAM, BERTRAND J., State University of New York, Upstate Medical Center, Syracuse
BROBERG, PATRICIA L., Brandeis University
BURNSTOCK, G., University of Illinois
BUTTERWORTH, FRANK M., University of Pennsylvania
BYERS, THOMAS J., University of Pennsylvania
CAMPBELL, JAMES WAYNE, Johns Hopkins University
CARLSON, ALBERT D., State University of Iowa
CHERNETSKI, KENT EUGENE, University of California
CURTIS, BRIAN A., Rockefeller Institute for Medical Research
DAVIDSON, MORTON, New York University Medical College
DUBNAU, DAVID, Columbia University
DUDEL, JOSEF, Johns Hopkins University
FAUST, ROBERT GILBERT, Princeton University
FILOSA, MICHAEL, Princeton University
GRAHAM, CHARLES EDWARD, Johns Hopkins University School of Medicine
GRIFFIN, DEAN H., American University
GUMP, DIETER W., Johns Hopkins University
GUTTMAN, BURTON S., Institute of Molecular Biology, University of Oregon
HURWITZ, CHARLES, VA Hospital, Albany
HUVER, CHARLES W., Yale University
JACKSON, JAMES A., Western Reserve University
KUPERMAN, ALBERT S., Cornell University Medical College
LAURIE, JOHN S., Tulane University
LIPICKY, RAYMOND JOHN, University of Cincinnati
DE LORENZO, A. J., Johns Hopkins University Medical School
NAGLER, ARNOLD L., New York University-Bellevue Medical Center
NARBAITZ, ROBERTO, Carnegie Institution of Washington and Universidad de Buenos Aires
ORKAND, RICHARD K., University of Utah
PEPE, FRANK A., University of Pennsylvania
POLGAR, GEORGE, University of Pennsylvania School of Medicine
POTTER, DAVID, Johns Hopkins University
REUBEN, JOHN P., College of Physicians and Surgeons, Columbia University
RUBIN, ARNOLD D., New York University College of Medicine
SCHAFER, DAVID G., New York University College of Medicine
SHEPHARD, DAVID, University of Chicago
SMITH, THOMAS G., JR., College of Physicians and Surgeons, Columbia University
SMYTH, THOMAS, JR., Pennsylvania State University
SUDDUTH, SOLON SCOTT, Johns Hopkins University School of Medicine
SWAMI, KARUMURI S., University of Pennsylvania
THEORELL, KLAS T. G., Karolinska Institutet, Stockholm, Sweden
TOBIN, MICHAEL, New York University Medical Center, Downstate
WARNER, ELDON D., University of Wisconsin

WERTHEIM, GUTA, Hebrew University
WHEELER, JAMES ENGLISH, Johns Hopkins University School of Medicine
WINICK, PAUL, Columbia University

Research Assistants, 1959

ALLEN, CONSTANCE, Massachusetts Eye and Ear Infirmary
ASHMAN, ROBERT F., Wabash College
ASHTON, FRANCIS T., University of Pennsylvania
ASTERITA, HARVEY L., New York University
BAIRD, SPENCER, Marine Biological Laboratory
BARNWELL, FRANKLIN H., Northwestern University
BARRON, EVELYN, Massachusetts Eye and Ear Infirmary
BERMAN, LAWRENCE J., Princeton University
BLUMSTEIN, JOYCE R., Albert Einstein College of Medicine
BOLEYN, BRENDA J., University of Rhode Island
BOUCK, G. BENJAMIN, Columbia University
BRANHAM, JOSEPH, Florida State University
BRAVERMAN, MAXWELL H., University of Illinois
BROOKS, KENNETH H., Indiana University
BUNIM, LESLEY S., Barnard College
CICAK, ANNA, Albert Einstein College of Medicine
CLARK, ELOISE E., University of California
CLARK, LYNNE G., Queens College
CONWAY, DOROTHY M., Rockefeller Institute for Medical Research
CORLETTE, SALLY L., Institute for Cancer Research
COUSINEAU, GILLES, University of New York
DELSON, ROZANNE, Massachusetts Institute of Technology
DINGLE, AL D., University of Illinois
DOOLITTLE, RUSSELL F., Harvard University
DOSS, DICKY E., American University
DUNSKY, MILTON H., Rockefeller Institute for Medical Research
EIGNER, ELIZABETH ANN, Massachusetts General Hospital
EIN, DANIEL, New York University-Bellevue Medical Center
ERSKINE, LOUISE, Institute for Muscle Research, Marine Biological Laboratory
ESPER, HILDEGARD, Columbia University
FELDHERR, CARL M., University of Pennsylvania
FIELDEN, ANN, University of Illinois
FINKEL, ARNOLD, New York University College of Medicine
FIORENTINO, EILEEN, Hahnemann Medical College
FISHER, FRANK M., JR., Purdue University
FORAN, ELIZABETH H., Smith College
FRIEDLER, GLADYS, Tufts Medical School
FRIEDMAN, LEONARD, Rutgers University
FULTON, CHANDLER M., Rockefeller Institute for Medical Research
GASSELING, MARY T., Marquette University
GEBHART, JOHN H., National Institutes of Health
GOLDFARB, DAVID, Johns Hopkins University
GOUDSMIT, ESTHER M., University of Michigan
GRIFFIN, JOE L., Princeton University
HAAS, FLORENCE ANNE, Western University Medical School
HALEY, BARBARA, Brandeis University
HALL, DONALD J., University of Michigan
HAMPSON, GEORGE RICHARD, Northeastern University
HANSON, FRANK E., JR., State University of Iowa
HASKELL, JUDITH ANN, Purdue University
HATHAWAY, RALPH R., Florida State University

HIKE, SALLY JAYNE, Mount Holyoke College
HILLMAN, CELIA A., Harvard University
HIMMELFARB, SYLVIA, University of Maryland School of Medicine
HOFFMAN, LARRY R., University of Texas
HOLSTEN, GEORGE H., III, Rutgers University
HOLT, CHARLES E., III, Massachusetts Institute of Technology
JACKSON, THOMAS JOHN, Lehigh University
JOHNSON, CHRISTINE A., Wheaton College
KAIGHN, MORRIS E., Massachusetts Institute of Technology
KORN, ROBERT WILLIAM, Indiana University
LAMONT, HAYES C., Columbia University
LEIGHTON, CHARLES, Colby College
LIBBIN, DICK, Bard College
LIPPERT, BYRON E., Indiana University
LONIGRO, NORMA, Seton Hill College
LORING, JANET, Harvard Medical School
LOVE, DAVID S., University of Colorado
MCCONNAUGHY, R. A., American University
MCGOWAN, BERNARD L., Johns Hopkins University
MCLAUGHLIN, JANE, Institute for Muscle Research, Marine Biological Laboratory
MANGUM, CHARLOTTE PRESTON, Vassar College
MAKINEN, PAULA, University of Minnesota
MALKOFF, DONALD B., University of Pittsburgh Medical School
MAVRIDIS, PARASKEVI J., Purdue University
MERRILL, CHARLOTTE F., Massachusetts Institute of Technology
MERSON, GERALD, New York University
MINGIOLI, ELIZABETH S., Harvard University
MOBBERLY, WILLIAM C., Tulane University
MORRISON, ROBERTA ANNE, Smith College
MOULE, JOHN WILLIAM, McMaster University
MOULE, MARGARET, McMaster University
MUELLER, HELMUT, Institute for Muscle Research, Marine Biological Laboratory
OTERO-VILARDEBO, LUIS, University of Puerto Rico
PALUBINKAS, BERTHA, College of Physicians and Surgeons, Columbia University
PERRY, BARBARA, Institute for Muscle Research, Marine Biological Laboratory
PHILPOTT, CHARLES W., Tulane University
REICH, MELVIN, Rutgers University
REUBEN, JOHN PHILLIP, University of Florida
ROBERTS, MARY LOU, Washington University Medical School
ROGERS, ANNETTE, North Carolina State College
ROSE, JEANNETTE, Bates College
ROSENBLUTH, RAJA, Columbia University
ROTHSTEIN, HOWARD, University of Pennsylvania
SATUREN, JANICE, State University of New York Upstate Medical Center at Syracuse
SCHROEDER, PAUL C., St. Peter's College
SCHUEL, HERBERT, University of Pennsylvania
SELLERS, RICHARD LEE, American University
SIEGEL, PAULA, University of Cincinnati
SIGER, ALVIN, Johns Hopkins University
SILKOVSKIS, IZOLDE, McMaster University
SIMMONS, JOHN E., Johns Hopkins University
SPENCER, JOYCE M., Harvard Medical School
STAUB, HERBERT W., Rutgers University
STOLL, LOUISE, Johns Hopkins University School of Hygiene
SWOPE, JULIA, Massachusetts General Hospital
SZENT-GYÖRGYI, EVA, Institute for Muscle Research, Marine Biological Laboratory
SZENT-GYÖRGYI, MARTA, Institute for Muscle Research, Marine Biological Laboratory

TEYAN, FRED, Albert Einstein College of Medicine
THOMAS, CYNTHIA, Massachusetts Eye and Ear Infirmary
VANLIEW, HUGH D., U. S. Navy, Bethesda
VANNORMAN, EARL, Princeton University
WAHBE, VERA, Kansas University
WATKINS, DUDLEY T., Oberlin College
YATES, LLOYD AUSTIN, University of Minnesota
YIP, CECIL C., McMaster University

Library Readers 1959

BALL, ERIC G., Professor of Biological Chemistry, Harvard Medical School
BAYLOR, MARTHA B., Investigator, Marine Biological Laboratory
BEIDLER, LLOYD M., Professor of Physiology, Florida State University
BODANSKY, OSCAR, Chief, Division of Metabolism and Enzyme Studies, Sloan-Kettering Institute
BROWN, DUGALD, Professor of Zoology, University of Michigan
BUTLER, ELMER G., Professor of Biology, Princeton University
CHASE, AURIN M., Associate Professor of Biology, Princeton University
CLARK, ELIOT R., University of Pennsylvania
COHEN, SEYMOUR S., Professor of Biochemistry, University of Pennsylvania School of Medicine
COLLIER, JACK R., Marine Biological Laboratory
FOERSTER, THEODOR, Professor of Physical Chemistry, Technische Hochschule, Stuttgart, W. Germany
FRIES, E. F. B., Associate Professor, City College of New York
GABRIEL, MORDECAI L., Associate Professor of Biology, Brooklyn College
GAFFRON, HANS, Professor of Biochemistry, University of Chicago
GINSBERG, HAROLD S., Associate Professor of Preventive Medicine, Western Reserve University
GOLDTHWAIT, DAVID A., Assistant Professor of Biochemistry, Western Reserve University
HUNTER, F. R., Professor and Head, Dept. of Biology, Univ. de los Andes, Bogota, Colombia
JACOBS, M. H., Professor Emeritus, University of Pennsylvania
KARUSH, FRED, Professor of Immunochemistry, University of Pennsylvania
KASHA, MICHAEL, Professor of Chemistry, State University of Florida
KLEIN, MORTON, Professor of Microbiology, Temple University School of Medicine
KOZLOFF, LLOYD M., Associate Professor of Biochemistry, University of Chicago
LEIGHTON, JOSEPH, Associate Professor of Pathology, University of Pittsburgh School of Medicine
LUBIN, MARTIN, Assistant Professor of Pharmacology, Harvard Medical School
LUDWIG, GEORGE D., Assistant Professor of Medicine, University of Pennsylvania
McDONALD, SISTER ELIZABETH SETON, Professor of Biology, College of Mt. St. Joseph on the Ohio
MINARD, FREDERICK, Research Biochemist, Abbott Laboratories
MOUL, EDWIN T., Associate Professor of Botany, Rutgers University
MUSACCHIA, X. J., Associate Professor in Biology, St. Louis University
NOVIKOFF, ALEX B., Research Professor, Albert Einstein College of Medicine
PULLMAN, BERNARD, Professor of Theoretical Chemistry, University of Paris, France
RHULAND, LIONEL E., Research Section Head, The Upjohn Company
ROCHOVANSKY, OLGA M., Research Assistant, Public Health Research Institute of New York City
ROOT, WALTER S., Professor of Physiology, College of Physicians and Surgeons, Columbia University
ROTH, FR. OWEN H., Associate Professor of Zoology, St. Vincent College
SCHLAMOWITZ, MAX, Associate Cancer Research Scientist, Roswell Park Memorial Institute
SERBER, BARBARA JO, Assistant Professor of Anatomy, New York University-Bellevue Medical Center
SONNENBLICK, B. P., Professor of Biology, Rutgers University
SULKIN, S. EDWARD, Professor and Chairman, Dept. of Microbiology, University of Texas, Southwestern Medical School

TRURNIT, HANS J., Senior Scientist, Research Institute for Advanced Study
WARNER, ROBERT C., Associate Professor of Biochemistry, New York University College of Medicine
WEIGLE, WILLIAM O., Assistant Research Professor, University of Pittsburgh School of Medicine
WHEELER, GEORGE E., Instructor in Biology, Brooklyn College
YNTEMA, CHESTER L., Professor of Anatomy, State University of New York, Upstate Medical Center
ZINN, DONALD J., Associate Professor of Zoology, University of Rhode Island

Students 1959

BOTANY

BROWN, MALCOLM, University of Texas
CHURCHILL, ALGERNON C., Harvard University
CORRELL, DAVID L., Michigan State University
EDWARDS, JACKIE L., University of Alabama
EHRLICH, DIANA LEE, College of the City of New York
FINDLEY, DAVIS L., University of Alabama
FLACH, MARY E., Vassar College
FOLDATS, ERNESTO, Universidad Central de Venezuela
FREDERICKS, WALTER W., Johns Hopkins University
GOLAS, MARY, Marquette University
KALIL, MILDRED, Wellesley College
KOOB, DERRY DELOS, Cornell University
MASON, CHARLES P., Cornell University
MILES, MARJORIE L., Acadia University
MORRIS, RUTH CAROL, Cornell University
NOLAN, RICHARD A., University of Nebraska
SHOR, BERNICE C., Rollins College
WAGNER, KENNETH A., College of William and Mary
WILLIAMS, RICHARD B., Harvard University
ZACHARIA, KURUVILA, Princeton University

EMBRYOLOGY

ASHMAN, ROBERT F., Wabash College
BAKER, JOHN R., University of Minnesota
BERGMANN, FRED H., Brandeis University
BIRKY, C. WILLIAM, JR., Indiana University
CORDES, EUGENE H., Brandeis University
CURTIS, JOSEPH C., Brown University
GIBLEY, CHARLES W., JR., Iowa State College
GRAND, THEODORE I., Brown University
GRINNELL, ALAN D., Harvard University
HARRIS, THOMAS M., University of North Carolina
HENNEN, SALLY H., Indiana University
HOLT, CHARLES E., III, Massachusetts Institute of Technology
KESSLER, DIETRICH, University of Wisconsin
LAWRENCE, IRVIN E., Kansas University
LESSUPS, ROLAND J., S. J., Johns Hopkins University
LEVINE, STEPHEN, Brandeis University
MERSON, GERALD, New York University Medical School
PIERCE, GORDON B., University of Pittsburgh
ROSE, IRWIN A., Yale University
SCHULER, MARGERY E., Wesleyan University

STEINBERG, SONIA NAOMI, Northwestern University
WHITTAKER, J. RICHARD, Yale University
YATES, ROBERT D., University of Alabama Medical Center

PHYSIOLOGY

ALVAREDO, FRANCISCO, New York University, College of Medicine
ANGELES, LETICIA, Tulane University
BENJAMIN, THOMAS, Amherst College
EISEN, JAMES, Emory University
GARRICK, MICHAEL, Johns Hopkins University
GILLESPIE, BARBARA, Radcliffe University
GOTTLIEB, ABRAHAM, New York University-Bellevue Medical Center
GREEN, MORRIS, University of Rochester
HAMILTON, MARY, Sloan-Kettering Institute
HANDLER, JOSEPH, University of Pennsylvania
HOMER, LOUIS, Medical College of Virginia
KALEY, GABOR, New York University
KEAN, EDWARD, University of Pennsylvania
KINSOLVING, CLYDE, Vanderbilt University
LIEBMAN, PAUL, Barnes Hospital
LUCHI, ROBERT, University of Pennsylvania
PLOTZ, PAUL, Harvard Medical School
PURPLE, RICHARD, Rockefeller Institute
ROSENBAUM, JOEL, Syracuse University
RYSER, HUGUES, Massachusetts General Hospital
SLAYMAN, CLIFFORD, Rockefeller Institute
THEORELL, HENNING, Karolinska Inst., Stockholm
TODARO, GEORGE, New York University College of Medicine
TOWNSEND, EDITH, McGill University
WALCH, CAROLYN, Johns Hopkins University
WEISS, CHARLES, Harvard University
WHITTENBURY, GUILLERMO, Harvard Medical School

INVERTEBRATE ZOOLOGY

ANDREW, OLIVER T., Franklin and Marshall College
BERCHMANS, SISTER ANN, St. Mary of the Woods College
BREBBIA, DANTE R., Fordham University Graduate School
BRENOWITZ, HARRY, Adelphi College
BUCKLEY, BROTHER WILLIAM, Fordham University
CHURCHILL, ALGERNON, Harvard University
CONROW, MARY M., Wilson College
CORRELL, DAVID, Michigan State University
DELONG, KARL T., Oberlin College
EDDY, JANE, Tufts University
EDWARD, BROTHER C., Fordham University
ELLISON, ESTHER, University of Minnesota
ENGLUND, PAUL, Hamilton College
EPEL, DAVID, University of California, Berkeley
FEIR, DOROTHY J., University of Wisconsin
FERGUSON, JOHN, Cornell University
GAGE, ELIZABETH M., Cushing Academy
GATES, DAVID A., Clark University
GOLDMAN, LAWRENCE, University of California, Los Angeles
GREENE, LAUREL E., Goucher College
GUTKNECHT, JOHN, University of North Carolina

HAYES, WILLIAM, University of Michigan
HENDERSON, OLIVER, JR., The Citadel
HUBER, SALLY A., Mt. Holyoke College
IZOWER, JACK, City College of New York
JONES, LYNNE A., Connecticut College
KRAUSE, HELEN, University of Massachusetts
LAFAUCI, GRACE, Wilson College
MANGUM, CHARLOTTE, Vassar College
MARZOLF, GEORGE, University of Michigan
MCDOWELL, SISTER MARGARET ANN, College of St. Mary of the Springs
MCWHINNIE, DOLORES J., DePaul University
MESCHER, SISTER ALMA L., University of Notre Dame
MOFFEY, ELIZABETH S., University of Michigan
MOULTON, JOHN, Hastings College and Clark University
NORBECK, BETTY, University of Minnesota
NORDLIE, FRANK, University of Minnesota
PROSSER, JANE ELLEN, Earlham College
RAPPAPORT, LUCINDA, Brandeis University
SEECK, MARGARET A., Oberlin College
SHAW, WILLIAM N., Bureau of Commercial Fisheries
SHOR, BERNICE, Rollins College
SIMPSON, MARGARET, Catholic University of America
STERNS, CAROL W., Peekskill, New York
STONG, CYNTHIA C., Wellesley College
THOMAS, CAROLINE, University of Vermont
VERRUSIO, A. CARL, Drew University
WILLIAMS, JUNARDEN, Northwestern University
ZIMMERMAN, WILLIAM, Princeton University
ZOTTOLI, ROBERT, Bowdoin College

ECOLOGY

ABBIATE, LORRAINE M., Douglass College
BACHMAN, ROGER W., University of Michigan
BIANCHI, CARLA F., Chatham College
BURKHOLDER, K. M., Emory University
DAVEY, TESSA, Mount Holyoke College
HAYWARD, GEORGE E., Drew University
PALMER, JOHN D., Northwestern University
PINCHOT, GIFFORD B., Johns Hopkins University
MCLAUGHLIN, ELLEN, University of North Carolina
SWEENEY, EDWARD F., Boston University
SWIFT, ELIJAH, Swarthmore College
TAYLOR, WALTER R., Johns Hopkins University
WATT, WALTON D., Dalhousie University
WHITELEY, GEORGE CO., The Hill School
WILLIAMS, ELSIE LOUISE, Goucher College

3. FELLOWSHIPS AND SCHOLARSHIPS, 1959

Lucretia Crocker Scholarships:
CHARLES P. MASON, Botany Course
JOHN D. PALMER, Ecology Course

Conklin Scholarship:
STEPHEN LEVINE, Embryology Course

Bio Club Scholarships:
DIANA LEE EHRLICH, Botany Course
JACK IZOWER, Invertebrate Zoology Course

4. Tabular View of Attendance, 1955–1959

	1955	*1956*	*1957*	*1958*	*1959*
Investigators—Total	250	304	326	410	427
Independent	162	184	186	208	215
Under Instruction	9	20	23	39	45
Library Readers	54	50	42	54	51
Research Assistants	25	50	75	114	116
Students—Total	148	140	139	138	134
Invertebrate Zoology	56	55	55	55	49
Embryology	30	28	27	22	23
Physiology	30	30	30	27	27
Botany	19	18	18	18	20
Ecology	13	9	9	16	15
Total Attendance	398	444	465	548	561
Less persons represented as both investigators and students		2	3	5	4
	398	442	462	543	557
Institutions Represented—Total	129	130	129	142	143
By Investigators	95	97	94	110	98
By Students	34	33	35	74	73
Schools and Academies Represented					
By Investigators	3	3	5		12
By Students	2	1	1	2	8
Foreign Institutions Represented					
By Investigators	8	9	11	20	29
By Students	6	6	5	6	9

5. Institutions Represented, 1959

Abbott Laboratories
A & M College of Texas
Adelphi College
Agricultural Research Center
Alabama, University of
Albert Einstein College of Medicine
American Heart Association
American University
Amherst College
Barnes Hospital
Bowdoin College
Brandeis University
Brooklyn College
Brown University
Bryn Mawr College
Buffalo, University of
California, University of
Carnegie Institution of Washington
Catholic University
Chatham College
Chicago, University of
Cincinnati, University of
City College of New York
Colby College
College of St. Mary of the Springs
College of William and Mary
Columbia University
Columbia University, College of Physicians and Surgeons
Connecticut College
Connecticut, University of
Cornell University
Cornell University Medical School
Cushing Academy
Department of the Interior
DePaul University
Drew University
Duke University
Earlham College
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Kansas University
Louisiana State University
Loyola College
Maine, University of
Manhattan College
Marquette University
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Massachusetts Eye and Ear Infirmary
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Michigan, University of
Minnesota, University of
Montefiore Hospital Research Institute
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Mt. St. Joseph, College of
National Institutes of Health
Nebraska, University of
New Hampshire, University of
New York State University College of Medicine at Syracuse
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New York University School of Dentistry
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Oregon, University of
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McGill University, Canada
McMaster University, Canada
University de los Andes, Bogota, Colombia
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Technical University, Darmtstadt, Germany

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Hebrew University, Israel
University of Milan, Milan, Italy
University of Tokyo, Japan
University of Puerto Rico, Puerto Rico
University of the Philippines, Philippines
Centro Investigaciones Biologicas, Madrid, Spain
Karolinska Institutet, Stockholm, Sweden
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University College of the West Indies, Jamaica, West Indies

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6. EVENING LECTURES, 1959

June 26
R. E. BILLINGHAM ..... "Studies on the Y chromosome antigen in rodents"

July 3
ALEXANDER FORBES ..... "The growth of physiology"

July 6
ALEXANDER FORBES ..... "Electrophysiology of color vision"

July 10
I. M. KLOTZ ..... "Protein hydration and behavior"

July 17
C. LADD PROSSER ..... "'The origin' after a century; prospects for the future"

July 24
SILVIO RANZI ..... "Protein differentiation during embryonic and larval development"

July 31
V. G. DETHIER ..... "Chemical sense of the blowfly and hunger"

August 7
CLAUDE A. VILLEE ..... "Interrelations of hormones and enzymes"

August 14
HUGO THEORELL ..... "Mode of action of enzyme-coenzyme complexes"

August 21

W. A. H. Rushton ..... "The retina is the net of a fisherman who catches quanta and barters them for information"

August 28

Eugene P. Odum ..... "The energy flow to the study of populations in nature"

7. Tuesday Evening Seminars, 1959

July 7

J. L. Griffin ..... "Isolation and chemical identification of the crystalline cytoplasmic inclusions in the large, free-living amebae"

Milton Fingerman ..... "Physicochemical characterization of chromatophorotropins in the crayfish, *Cambarellus shufeldti*"

William H. Johnson and Andrew G. Szent Györgyi ..... "The molecular basis for the 'catch mechanism' in molluscan muscles"

July 14

James D. Eisen ..... "A study on the physiology of the predator-prey relationship existing between *Paramecium aurelia* and *Didinium nasutum*"

Wolfgang Wieser ..... "Growth, metabolism and coexistence in marine nematodes"

Ralph A. Lewin ..... "Uptake of strontium by *Syracosphaera*"

July 21

A. B. Novikoff ..... "Lysosomes in the physiology and pathology of cells"

J. R. Collier ..... "Localization and synthesis of ribonucleic acid in the development of *Ilyanassa obsoleta*"

G. G. Holz, Jr. and C. C. Speidel ..... "Mating behavior of x-rayed *Tetrahymena pyriformis*." Motion pictures

C. Fulton ..... "Polarized tissue movement in hydroid regeneration." Motion pictures

July 28

Philip Person, Jay W. Lash and Albert Fine ..... "Myoglobin and cytochrome oxidase in odontophore cartilage of Busycon"

W. Troll, S. Belman and N. Nelson ..... "Aromatic amine metabolism and bladder cancer"

Paul S. Galtsoff and D. E. Philpott ..... "Ultra structure of the spermatozoon of the oyster"

August 4

Vincenzo Leone ..... "Some structures found in electron microscopic pictures of an amphibian tumour"

Alfred W. Senft ..... "Ultrastructure of the human parasite, *Schistosoma mansoni*"

GEORGE W. DE VILLAFRANCA AND DELBERT E. PHILPOTT ..... "A study of the fine structure of skeletal muscle from *Limulus polyphemus*"

August 11

MAURICE M. RAPPORT ..... "Present status of the problem of plasmalogen structure"

ERIC G. BALL ..... "On the mode of action of insulin"

WALTER S. VINCENT AND ELYANE BALTUS ..... "Incorporation of isotopic label into RNA: synthesis or terminal addition?"

August 18

L. V. HEILBRUNN ..... "The action of glycerol on protoplasm"

WALTER L. WILSON AND K. S. SWAMI ..... "Electrophoretic studies on protoplasm"

FRANCIS T. ASHTON ..... "Germinal vesicle breakdown in the eggs of Spisula and Hydroides"

R. D. ALLEN ..... "Polarized optical studies on Ameba"

F. CHILD ..... "Isolation and analysis of cilia"

8. MEMBERS OF THE CORPORATION, 1959

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Saunders, Mr. and Mrs. Lawrence
Shiverick, Mrs. Arthur
Sinclair, Mr. and Mrs. W. Richardson
Speidel, Dr. and Mrs. Carl
Stockard, Mrs. Charles R.
Stone, Mr. and Mrs. Leo
Stone, Mr. and Mrs. S. M.
Straus, Dr. and Mrs. Donald B.
Swift, Mr. E. Kent
Swope, Mr. and Mrs. Gerard, Jr.
Swope, Miss Henrietta
Tompkins, Mr. and Mrs. B. A.
Webster, Mrs. Edwin S.
Whiteley, Miss Mabel W.
Wickersham, Mr. and Mrs. James H.
Wilhelm, Dr. and Mrs. Hilmer J.
Williston, Miss Emily
Wilson, Mrs. Edmund B.
Wolfinsohn, Mrs. Wolfe

V. REPORT OF THE LIBRARIAN

During 1959, forty-eight new journals were acquired making a total of 1665 currently-received titles. Of these, there were 484 (12 new) Marine Biological Laboratory subscriptions, 638 (9 new) exchanges and 184 (5 new) gifts; 100 (7 new) were Woods Hole Oceanographic Institution subscriptions; 199 (12 new) were exchanges and 60 (3 new) were gifts. Between the years 1950 and 1959, 439 new journals were obtained with initial date of publication, in each case, falling within that period.

The Laboratory purchased 92 books (15 of these from the Montgomery Memorial Fund), received 119 complimentary copies (7 from authors and 112 from publishers) and accepted 43 miscellaneous gifts. The Institution purchased 50 books and received 6 as gifts. The total number of books accessioned amounted to 310.

Through purchase, exchange and gift the Laboratory completed 10 journal sets and partially completed 15. The Institution completed 6 sets and partially completed 6. There were 5,629 reprints added to the collection, of which 1772 were of current issue.

At the close of the year there were 76,073 bound volumes and 212,627 reprints.

The Library mailed out on inter-library loan 384 volumes and borrowed 72. About 900 volumes were bound, as well as 85 pamphlets.

Dr. E. V. Cowdry presented his large collection of reprints to the Library, of which 2000 were added to the shelves. Among his collection there were several journal numbers which filled in gaps of long standing. Dr. F. A. Hartman presented a collection which will be processed in 1960. Also, gifts of reprints and books were received from Dr. H. W. Kaan, Dr. P. W. Whiting and Mrs. A. R.

Memhard. Dr. W. R. Amberson donated a long series of the serial entitled "Onderzoekingen gedaan in het Physiologisch Laboratorium der Rijksuniversiteit te Utrecht," as well as several books. To each of these generous friends the Laboratory wishes to extend grateful thanks for the valuable literature acquired by the Library.

Two foreign institutions benefited from the collection of duplicates, namely, the National Institution of Oceanography in England and the Marine Biological Laboratory at Helsingör, Denmark.

During 1959, the Staff noticed a considerable increase in the use of the Library during the winter months. This is very gratifying, as it indicates further year-round use of the Library facilities, as Woods Hole becomes more and more a scientific research center.

Respectfully submitted,
DEBORAH L. HARLOW,
Librarian

VI. GENERAL BIOLOGICAL SUPPLY HOUSE, INC.

It would seem that a short resumé of the history of "Turtox" would be of interest to the members of the Corporation at this time.

In 1913, Morris Wells was a graduate student in the Department of Zoology of the University of Chicago. Dr. Frank R. Lillie was Chairman of the Department, and also President of the Marine Biological Laboratory. Prior to this, Mr. Wells had taught biology for one year in a high school in Kansas. This experience made him realize that biology teachers needed aid in obtaining material for instruction. In 1914, he and his wife prepared a one-page mimeographed sheet, listing slides and other material, which was mailed to a list of biology teachers. Several orders for $1.00 each were received, which were processed in the cellar of Mrs. Wells' parents' home. Mr. Wells received his Doctor's degree in 1915, and accepted a position as Instructor under Dr. Frank R. Lillie, at the University of Chicago. He was promoted to the rank of Assistant Professor three years later. By 1918, Dr. Wells realized he could no longer do justice to his teaching, and take care of his growing business. He discussed this with Dr. Frank Lillie. Dr. Lillie suggested that if Dr. Wells wished to devote all his time to furnishing biological supplies to teachers, he might interest his brother-in-law, Mr. Charles Crane, in the business. A corporation was formed, under the laws of the State of New York; Dr. Frank Lillie was Chairman of its Board of Directors for many years. Mr. Crane purchased 51% of the stock of the new company, and turned it over to the Marine Biological Laboratory as a gift. The amount involved was $10,000.

In 1920, due to the growth of the business, the General Biological Supply House increased their stock by $5000, and the M. B. L. purchased one-half of the new issue for $2500.

In 1921, the Treasurer's report of the M. B. L. shows the entire holdings of General Biological Supply House stock held by M. B. L. listed at $12,700. This figure includes 51% of the voting stock. The purpose of this arrangement was to keep the business in the control of a scientific institution, which would prevent any

possible future hazards of the business being run just for the profit of the private owners of the stock. Dr. Wells insisted from the start that the most important functions of the new company were to help biology teachers get material, and pass on to them information concerning new techniques.

The new company was called "The General Biological Supply House Inc." and Dr. Wells coined the word "Turtox," with a design of a turtle holding up the world.

Information about the new company during the first five years is not available, except that 8% of the par value of the stock was paid in dividends during that period. From 1923 to the present date, a period of 36 years, the M. B. L. has received $476,144 in dividends and its stock interest in the company at this time is worth well over $500,000.

In 1919 Dr. Wells had a student in one of his courses who seemed to have potentialities. Dr. Wells suggested that he continue his courses in fundamental biology, and take courses in commerce. This student followed Dr. Wells' suggestion, and took his degree in Business Administration. He worked part time, as a student, for "Turtox," and became a full time employee when he graduated. Dr. Wells was ill for long periods, and this man was elected Vice President. When Dr. Wells died, he became President, a position he still holds. He is, of course, Mr. C. Blair Coursen. Part of Blair Coursen's work until recently was to edit the magazine, "Turtox News"; Mrs. Shepherd, who worked with Mr. Coursen for several years, is the present editor. "Turtox News" devotes more than half its pages to non-advertising material. Secondary school biology teachers find it "good reading."

In 1955, General Biological Supply House moved to a new building, especially designed for their operation. This building has proved to have been a wise investment.

In 1957, the Board of Directors established the "Turtox Scholarship." Any American citizen who is currently, or has been, enrolled in a graduate school of biology is eligible. The award is based upon evidence bearing upon the promise of the applicant as a prospective teacher and research scholar. The stipend is $5000 per year—one of the largest scholarships available. The Scholarship Committee consists of Dr. Frank A. Brown, Jr., Chairman, Dr. Philip B. Armstrong, Dr. C. E. Olmstead, Dr. D. P. Rogers and Dr. S. Meryl Rose.

In preparing this report, I wish to acknowledge help from Mr. C. Blair Coursen, Mrs. Edith Wells, Dr. Winterton C. Curtis, Mr. Homer P. Smith and the Librarian, Mrs. Deborah L. Harlow.

The successful operation of the General Biological Supply House Inc. reflects the management of its President, C. Blair Coursen, ably assisted by the Vice President, Arold Blaufuss, the Export Manager, Charles Coursen, Jr., and the Assistant to the President and Editor of "Turtox News," Mrs. Ruth L. Shepherd. They, together with a group of well trained and loyal employees, have rendered a unique service to teachers of biology throughout the world. Thus, the original purpose of its founder, Dr. Morris Wells—to give aid and assistance to biology teachers—is being carried out.

Respectfully submitted,
C. Lloyd Claff

THE EFFECT OF SALINITY ON GROWTH OF GYMNODINIUM BREVE DAVIS

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Field observations have established the close physical association of mass mortalities of marine animals in the Gulf of Mexico with high concentrations of the non-thecate dinoflagellate *Gymnodinium breve* Davis (Davis, 1948; Galtsoff, 1948, 1949; Gunter *et al.*, 1948; Wilson and Ray, 1956, among others). With the development of satisfactory culture media (Wilson and Collier, 1955) and successful methods for growing the organism in the absence of bacteria (Ray and Wilson, unpublished results), more definitive study of this association became possible. Subsequently Ray and Wilson (1957), and Starr (1958) conclusively demonstrated the toxicity of *G. breve* to fishes.

The catastrophic manifestations of naturally-occurring *G. breve* blooms have attracted considerable attention. The sporadic nature of the outbreaks has stimulated particular interest in possible relationships between environmental factors and these "red tides." In this regard, various investigators, drawing from relatively sparse field data, have postulated the importance of salinity, dissolved nutrients, and meteorological conditions (see Ryther, 1955, for review). This report deals with the effect of salinity on the growth *in vitro* of *G. breve* and compares these findings with the field observations of other workers.

The technical assistance of Mrs. Alice Kitchel is gratefully acknowledged.

Materials and Methods

Bacteria-free cultures (10-ml. aliquots in 16 × 125 mm. screw-capped Pyrex tubes) were employed throughout this work. These tubes, together with the flasks and pipettes used in medium preparation or inoculation, were rigorously cleaned before each use. The cleaning routine, found by Ray and Wilson (unpublished results) to be an important factor in the successful culturing of this organism, included the use of a detergent, hot 10% nitric acid, and repeated rinses in tap water and distilled water. In an additional step, culture tubes were filled with, and inoculation micropipettes immersed in, triple-distilled water and autoclaved for 15 minutes at 15 p.s.i.

All control tubes contained a completely synthetic medium (Table I), compounded by one of us (W.B.W.) which supported good growth of *G. breve*. The medium in experimental tubes differed from control medium only in major salt content (NaCl, $MgSO_4$, $MgCl_2$, $CaCl_2$ and KCl). In varying salinity these major constituents were varied proportionally, thus producing no change in the ion ratios.

Pasteur capillary pipettes were used to inoculate each of a series of tubes of medium with 100–200 cells from well-established cultures of *G. breve*. Two or three cultures were used to inoculate each experiment. Equal numbers of replicates from each salinity group were inoculated with a given culture so that physiological differences between inocula would not bias results. After inoculation, the new cultures were maintained at a temperature of 26–27° C., and illuminated by two 30-watt "standard cool white" fluorescent lights two to three inches from the culture tubes.

Glassware and media were sterilized by autoclaving at 15 p.s.i. for 15 minutes. After inoculations were completed, two bacterial sterility tests were conducted for each culture used as inoculum. These tests involved pipetting 1 ml. of the inoculum culture into each of two culture tubes, one containing 10 ml.

TABLE I

Gymnodinium breve *culture medium*

NaCl*	29.0 gm.	KNO_2	1.0 mg.
$MgSO_4 \cdot 7H_2O$	6.0 gm.	KNO_3	1.0 mg.
$MgCl_2 \cdot 6H_2O$	4.5 gm.	Thiamine†	1.0 mg.
$CaCl_2$	0.7 gm.	Vitamin B_{12}†	1.0 μg.
KCl	0.6 gm.	Biotin†	0.5 μg.
Tris(hydroxymethyl)-aminomethane**	20.0 mg.	Sulfide solution‡	5.0 ml.
$Na_2S \cdot 9H_2O$	3.0 mg.	Metals solution§	20.0 ml.
K_2HPO_4	1.0 mg.	Triple-distilled water	1000 ml.

* A. R. grade, recrystallized from triple-distilled water by the addition of C. P. HCl. All other inorganic compounds were C. P. or A. R. grade.

** Fisher Scientific Co. Added as 50 ml. of a stock solution adjusted to pH 8.2 by the addition of HCl.

† Nutritional Biochemicals Corp.

‡ Five ml. of this solution (derived from van Niel, 1931) contains: NH_4Cl, 1.0 mg.; $NaHCO_3$, 1.0 mg.; $Na_2S \cdot 9H_2O$, 0.8 mg.; KH_2PO_4, 0.5 mg.; $MgCl_2 \cdot 6H_2O$, 0.2 mg.

§ Twenty ml. of this solution contains: (Ethylenedinitrilo)tetraacetic acid disodium salt (Eastman Kodak Co.), 3.0 mg.; Mn as $MnCl_2 \cdot 4H_2O$, 0.2 mg.; Rb as RbCl, 0.2 mg.; Al as $AlCl_3 \cdot 6H_2O$, 0.1 mg.; Co as $CoCl_2 \cdot 6H_2O$, 0.1 mg.; Cs as CsCl, 0.1 mg.; B as H_2BO_3, 0.1 mg.; Se as H_2SeO_3, 0.1 mg.; Cr as K_2CrO_7, 0.1 mg.; Mo as $Na_2MoO_4 \cdot 2H_2O$, 0.1 mg.; Sr as $SrCl_2 \cdot 6H_2O$, 0.1 mg.; Ti as TiO_2, 0.1 mg.; Zn as $ZnCl_2$, 0.1 mg.; Zr as $ZrOCl_2 \cdot 8H_2O$, 0.1 mg.; Ba as $BaCl_2$, 0.02 mg.; Cd as $CdCl_2 \cdot 2\frac{1}{2}H_2O$, 0.02 mg.; Cu as $CuCl_2$, 0.02 mg.; Fe as $FeCl_2 \cdot 4H_2O$, 0.02 mg.; Ce as $(NH_4)_2Ce(NO_3)_6$, 0.02 mg.; V as NH_4VO_3, 0.02 mg.; Ni as $NiCl_2 \cdot 6H_2O$, 0.02 mg.; Rh as $RhCl_3$, 0.02 mg.; Ru as $RuCl_3$, 0.02 mg.; Sn as $SnCl_2 \cdot 2H_2O$, 0.02 mg.

of peptone sea water broth, the other a 10 ml. peptone sea water agar slant (Spencer, 1952).

Growth of the dinoflagellate was estimated by visual examination of the tubed cultures with the aid of a stereoscopic miscroscope, using 9× magnification for most cultures, and 18× or 27× when populations were low. Eleven graded population categories were adopted and "peak population" arbitrarily defined to include the top three. A rough calibration of this method was carried out by making estimates and actual cell counts from the same cultures, and comparing results. This check was conducted on four occasions, and, in all, 99 test cultures were examined by both methods. Cultures showing peak population by visual

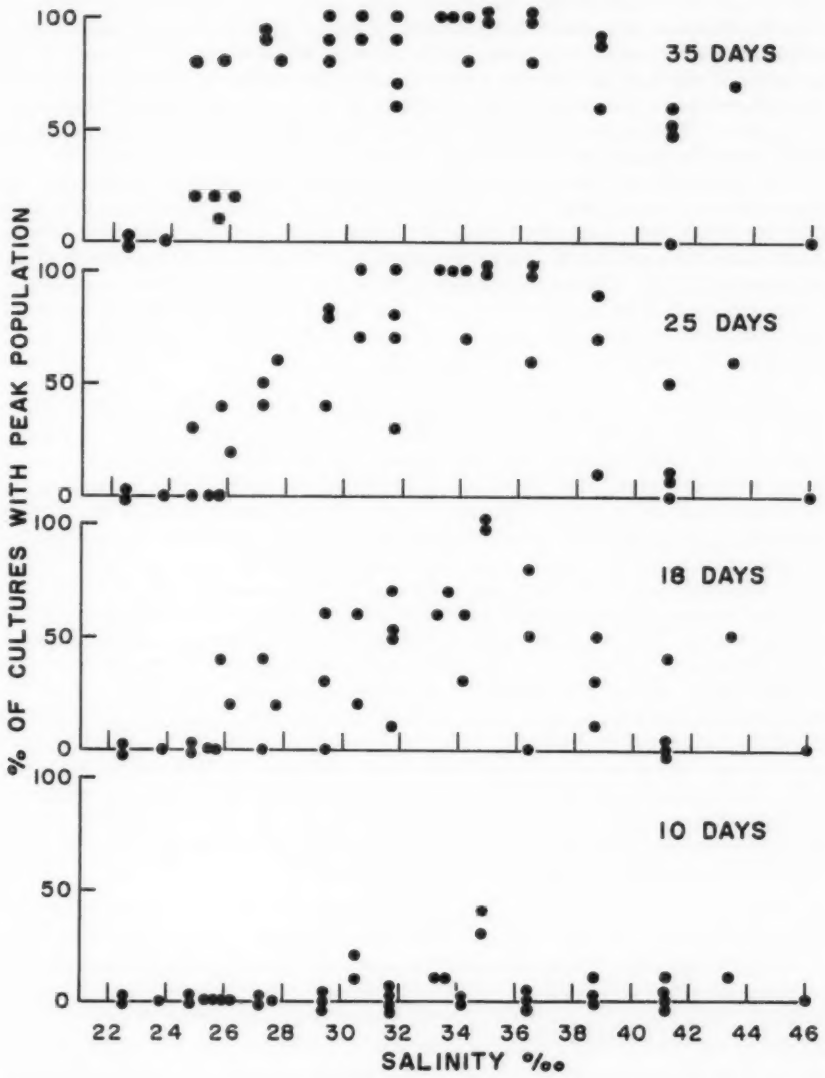

FIGURE 1. Rate of peak population development of *Gymnodinium breve* at various salinities. Each point is based on 10 replicate cultures.

estimate never proved to have fewer than 750 cells per ml., and usually contained from one thousand to several thousand cells per ml. Although sacrificing a degree of quantitative accuracy, the visual estimate method was selected because of the ease, speed, and lack of bacterial contamination with which it could be performed. All cultures were examined by this method at 4, 10, 18, 25, and 35 days after inoculation. The five-week period was considered adequate, since cultures seldom grow after five weeks post-inoculation.

Five experiments were conducted, each having nine salinity levels and ten replicate tubes at each level. The first experiment had the widest salinity range (6.3, 8.4, 11.9, 13.7, 17.8, 18.3, 25.8, 31.7, and 41.1‰). The four later experiments were designed for a salinity range of 22.5 to 41.1‰ with intervals of approximately 2.3‰. However, due to technical error, the last two experiments had ranges of 25.4 to 36.3‰ and 23.8 to 46.0‰ and somewhat irregular intervals.

Results

Cultures of *G. breve* grew well throughout a salinity range of 27 to 37‰ (Fig. 1). Within this range, at least 80% of replicate cultures reached populations of 750 cells or more per ml. during the five-week experimental period. More variable results and generally poorer growth occurred at salinity levels immediately adjacent to the optimal range. No instances of optimum growth occurred at less than 24‰ or more than 44‰. Some indication of comparative growth rates may also be obtained from Figure 1. It is apparent that cultures reach high populations more rapidly within the optimal range (27 to 37‰).

Some organisms survived throughout a salinity range of 22.5 to 46.0‰. From 24.8 to 46.0‰, 91% of cultures contained living cells at the end of the five-week observation period. There was no indication of reduced survival at the extremes of this range. Below this range the incidence of survival was lower; at 23.8‰ the organism survived in only one of 10 replicate cultures, and at 22.5‰ 10 of 20 replicates contained surviving cells. No instances of five-week survival were noted at any of the tested salinity levels below 22.5‰. At 18.3 and 17.8‰ three and two, respectively, of the 10 tubes in each group contained a few live organisms 10 days after inoculation, but no survivors were found eight days later. Media with salt concentrations of 13.7‰ or less contained no visible live *G. breve* one day after inoculation or thereafter.

Discussion

Reports discussing dinoflagellate salinity tolerances indicate a general euryhaline tendency for the group (Biecheler, 1952; Braarud, 1951; Braarud and Rossavik, 1951; Braarud and Pappas, 1951; Nordli, 1953; Provasoli *et al.*, 1954). Of the 10 forms studied by these workers, only one, *Peridinium balticum*, gave evidence of a stenohaline character (Provasoli *et al.*, 1954). Our results suggest that the range of salinity tolerated by *G. breve* is narrower than that of the dinoflagellates studied by other workers.

The literature relating to the occurrence of *G. breve* in the field contains a variety of statements concerning salinity. Fritsch (1956), in a general statement concerning Dinophyceae, noted (p. 664): "In the oceanic plankton the naked

types abound, while the neritic plankton is far richer in armoured forms." Commenting on salinities existing during the 1946–47 series of red tide outbreaks along the Florida coast, Gunter *et al.*, (1948) state (p. 320): "Comparing these results with those of normal sea water at various stations in south Florida . . . one may conclude that the . . . salinities are not abnormal." This statement is based largely on salinity values of 35.5 to 37.0‰.

Other workers report the occurrence of *G. breve* blooms in Florida waters having salinities below those associated with the open Gulf. Hela (1956) concluded that 31 to 34‰ represented the most favorable salinity range for blooms. Odum *et al.* (1956) found the organism most frequently in waters having salinities of 33 to 35‰. Observations of *G. breve* blooms by Ketchum and Keen (1948) and Chew (1953) include salinity values with ranges of 32.5 to 33.2‰ and 33.49 to 34.50‰, respectively.

Slobodkin (1953) suggested (p. 151), ". . . red tides require a discrete mass of water of relatively low salinity." He pointed out that the salinity difference

TABLE II

Salinity and incidence of G. breve *in Florida west coast waters (computed from Finucane and Dragovich, 1959)*

Salinity range (‰)	Samples collected	Samples positive for *G. breve*		Samples containing *G. breve* in lethal concentrations (250 or more/ml.)	
		Number	Per cent	Number	Per cent
39.00–40.99	107	0	0	0	0
37.00–38.99	1063	27	2.5	0	0
35.00–36.99	4010	397	9.9	15	0.4
33.00–34.99	1473	359	24.4	53	3.6
31.00–32.99	379	55	14.5	1	0.3
29.00–30.99	179	21	11.7	2	1.1
27.00–28.99	114	10	8.8	0	0
25.00–26.99	77	6	7.8	0	0
23.00–24.99	45	4	8.9	1	2.2
21.00–22.99	37	3	8.1	0	0
0.00–20.99	401	0	0	0	0

between a water mass and the surrounding water tends physically to preserve the identity of the mass. The inference was that the duration of water masses "physiologically suitable" for *G. breve* blooms determined the population levels which could be reached. On the other hand, Ryther (1955), reviewing red tide conditions as reported by Ketchum and Keen (1948) and Chew (1953), pointed out (p. 401) that ". . . where such measurements have been made, the salinity in patches of red water does not appear to be significantly lower than that of the surrounding, clear ocean water."

The obvious differences in the results and conclusions of these field studies are probably related to the small number of observations made in each case. Even so, the noted salinity values fall within the optimum range suggested by our culture studies.

The frequency of *G. breve* occurrence was determined for more than 7000 water samples of known salinity (Table II), using the field data of Finucane and Dragovich (1959). The incidence of the organism was significantly higher in salt concentrations lower (33.00 to 34.99‰) than those usually encountered in waters of the open Gulf (35.00 to 36.99‰). This observation was even more striking when only the potentially fish-killing population densities (approximately 250 or more cells per ml.) were considered.

Reduced salinity *per se* was apparently not a biologically essential factor for good growth of *G. breve* in culture. In the field the association of high incidence of this flagellate with salinities slightly below those of undiluted Gulf water may be due to the presence of dissolved nutrients in land drainage of the Florida west coast (Wilson and Collier, 1955). Water with salt *and* nutrient concentrations conducive to growth of dense populations of this organism may only occur in areas which receive nutrient-rich fresh water. The physical effect of salinity differences on the "life expectancy" of water masses as discussed by Slobodkin (1953) may represent another way in which salinity affects the population of *G. breve*. However, evidence is lacking on this point.

Finucane and Dragovich's (1959) data also show a few instances of the occurrence of *G. breve* at salinity levels between 21 and 25‰. The presence of the organism under these conditions is noteworthy, although the relatively few water samples in this range necessitate wide confidence limits for the per cent incidence values obtained. The long survival of this flagellate in culture at salinity levels inhibitory to growth suggests that it may also exist in the field long after salinity conditions have ceased to be favorable. Furthermore, salinity decreases in the field are probably more gradual than those of our experimental conditions, and may permit more acclimation.

In regard to *G. breve* distribution, our results indicate that high salinity may limit growth of this organism only in areas in which high evaporation with low runoff and mixing cause salt concentrations to rise well above offshore Gulf values. At the other end of the range, however, below 24‰ salinity may be a limiting factor in estuarine environments. Slobodkin (personal communication cited by Ryther, 1955) suggested that between outbreaks "seed populations of this organism are maintained in the brackish to freshwater regions of the Florida Everglades." In opposition to this view, our findings show that marine waters may present the most favorable environment for subsistence. Furthermore, recent field data (Finucane and Dragovich, 1959) indicate a lower incidence of this flagellate in Florida west coast embayments than in the Gulf during non-red tide periods.

When considered with nutritional requirements, the relatively stenohaline character of *G. breve* may explain the comparatively localized distribution of dense populations of this protist.

Summary

1. Bacteria-free *Gymnodinium breve* were exposed to media with salinity values ranging from 6.3 to 46.0‰; the best growth occurred between 27 and 37‰. These results indicate *G. breve* to be a relative stenohaline dinoflagellate.

2. Field evidence associates high incidence of dense populations with salinity levels a few parts per thousand below those of the offshore waters of the Gulf of Mexico. Our results suggest that this field distribution does not represent a salinity requirement *per se,* since salt concentrations equivalent to those of the open Gulf did not inhibit growth of this organism in culture.

3. No instances of optimal growth occurred in culture media with salinity levels of 24‰ or less. Under equivalent estuarine conditions salinity may be a limiting factor in the natural distribution of *G. breve.*

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A MAGNETIC COMPASS RESPONSE OF AN ORGANISM[1]

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Previously reported studies (Brown, Brett, Bennett and Barnwell, 1960; Brown Webb and Brett, 1960) have established beyond reasonable doubt that mud-snails, *Nassarius obsoleta,* are able to perceive and exhibit quantitative alterations in orientational responses to a change in the magnetic field strength amounting to about a 10-fold increase over the earth's natural one. In these studies the experimentally increased field appeared to effect predominantly the same kind of response, whether the field direction remained unaltered or was abruptly rotated through 90° in a horizontal plane. The size of the response to the change in strength of field, and in some measure also the character of the response, were clearly functions of phases of both solar and lunar periods. The response was one of graded amounts of clockwise or counterclockwise turning in the magnetic field.

If orientation in a magnetic field were to be a phenomenon of a useful nature in spatial orientation and navigation, it would be expected that the organism would exhibit a capacity to distinguish the directions of lines of magnetic force as indicated by differential responses to fields at right angles to one another. Suggestion that this might be the case has been reported (Brown, Webb, Bennett and Barnwell, 1959). The following is an analysis to determine whether the snails actually possess such a capacity to respond differentially.

Method

The apparatus and methods for obtaining the data have been reported earlier (Brown, Brett, Bennett and Barnwell, 1960). In essence, the experiments comprised permitting snails to emerge from a magnetic-south-directed, straight, narrow corridor into a constant, symmetrical field provided with a grid such that the mean amount of right or left turning during the initial 3 cm. of free movement could be assayed. Each experimental series consisted of two samples of ten passages in the earth's field, two samples of ten in an experimentally increased field oriented as the earth's, and two samples of ten in an increased field rotated 90° clockwise from the natural. The order of the groups of ten in the series of 60 was scrambled except the first three groups of ten in nearly every instance consisted of one with each field, as did also the second of the three groups of ten. Between June 28 and August 29 a total of 564 series of 60 snail runs was obtained between the hours of 5 AM and 9 PM. Though only 17 hours of the solar-day were represented by the data, all hours of a lunar day and all days of a natural synodic month were represented. The number of series for single solar-day hours ranged from 9 to 50, for single lunar-day hours from 5 to 22 and for the single

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days of a natural semi-monthly period (15-day periods synchronized to new and full moon) from 3 to 37.

Results

A daily rhythm was found in the relative effectiveness on the orientation of the snails of the two magnetic fields oriented at right angles to one another. This

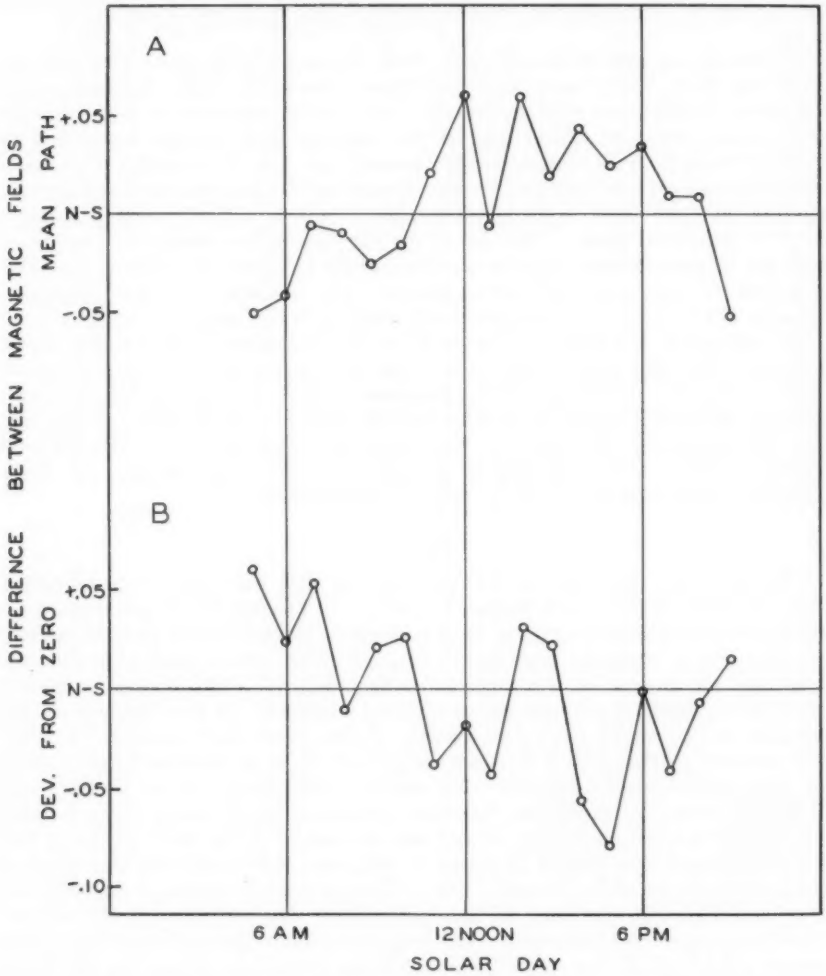

Figure 1. A. The drift through the solar-day of the effectiveness of the E-W magnet field relative to the N-S field in producing mean path difference from controls. B. Same as for A, except for being relative effectiveness in producing dispersion of paths from zero, irrespective of sign.

was demonstrated first for the amount of induced turning of snails in the experimental magnetic fields expressed as difference between the responses of the snails in these fields and the controls in the same series. In Figure 1A is shown the systematic fluctuation in effectiveness of the East-West (E-W) magnetic field relative to the North-South (N-S) field. In the early morning (5–6 AM) the E-W field is the more effective in producing counterclockwise turning of the snails; this is followed by a gradual drift to a time shortly after noon when the N-S field is more effective in producing comparable turning, whereafter, there is a gradual return to a greater effectiveness of the E-W field in the evening. A correlation between the relative effect of the two magnetic fields and the hour angle of the sun expressed as deviation from 2:30 PM (+37.5°) yielded a coefficient of 0.794 ($N = 17$; $P < 0.001$).

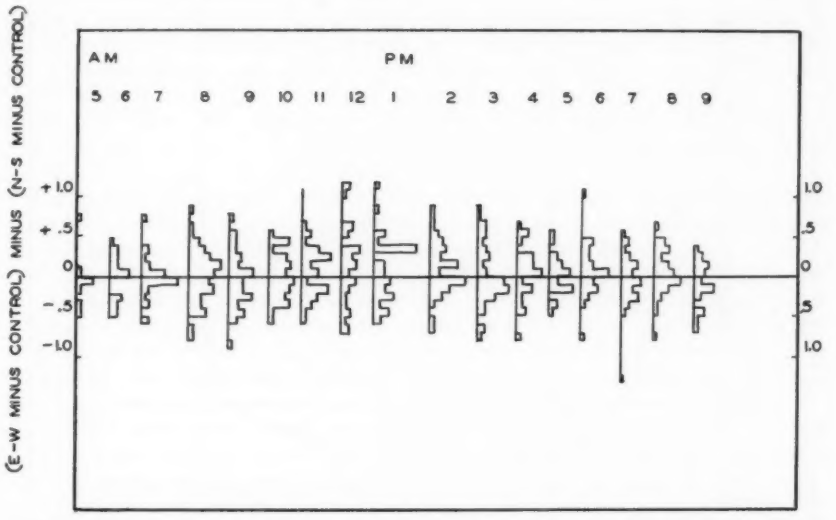

FIGURE 2. Frequency distributions, as a function of hour of day, of the differences between effectiveness of the experimental E-W and N-S fields in altering mean path from the control one.

A comparable daily rhythm was also seen in the difference between the responses to the two magnetic fields in effecting alterations in the total dispersion of pathways, both clockwise and counterclockwise. In Figure 1B it is seen that the E-W field is more effective in producing dispersion of pathways in the early morning, whereas the N-S field tends gradually to become more effective until late afternoon when the relationship seems to reverse again. When this relationship of the two magnetic fields is examined as a linear correlation with the hour angle of the sun expressed as deviation from 4:30 PM (+67.5°), a coefficient of 0.664 is found ($N = 17$; $P < 0.005$).

A daily rhythm in the response as measured by dispersion of paths, or total turning response to magnetic field, whether clockwise or counterclockwise, is perhaps more evident in Figure 2, in which the frequency distributions of the

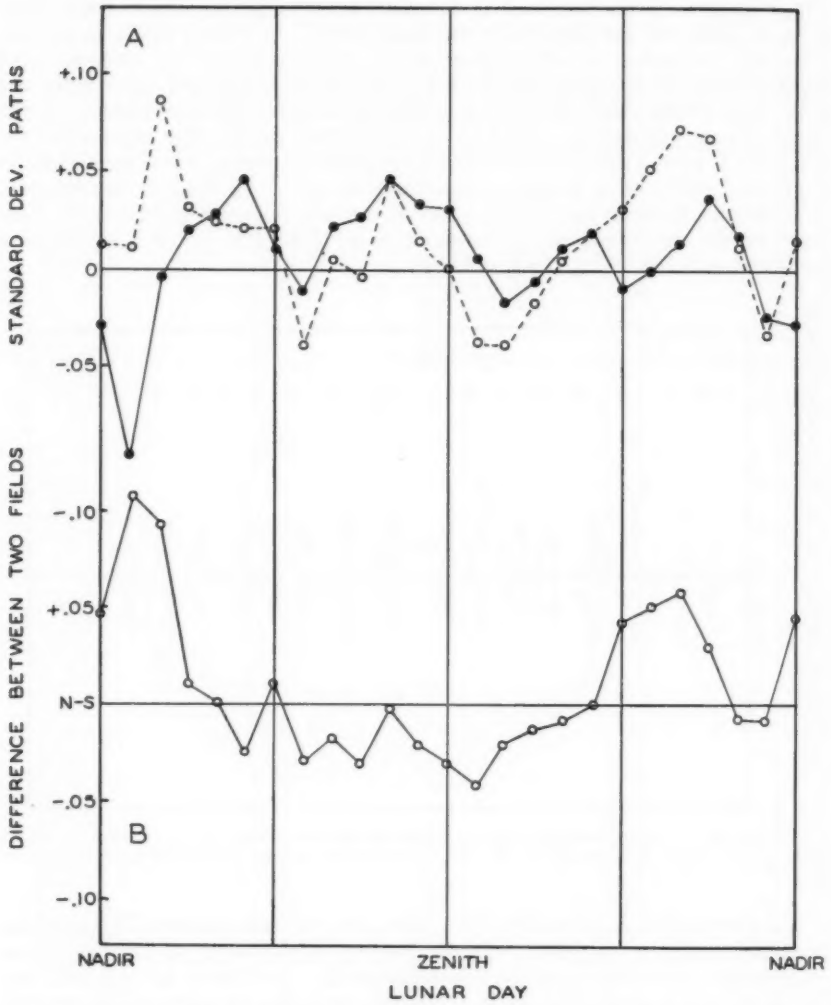

FIGURE 3. A. The difference of the standard deviations for snails in the E-W (dashed line) and N-S (solid line) fields from standard deviation of the controls as a function of hour of lunar-day. B. Drift through the lunar-day of the effectiveness of the E-W field relative to the N-S one in altering standard deviation from that of the controls. Points are calculated from three-hour grouped data.

differences between the responses to the two magnetic orientations are shown as functions of the hours of the solar day. Study of this figure reveals a tendency towards unimodality of distributions in the early morning and evening, with greater dispersion and a definite tendency toward bimodality during the middle of the day. This daily difference between the responses to the two magnetic fields can be demonstrated to be statistically probable beyond reasonable doubt by comparing standard deviations of the distributions. For example, the values for 5–10 AM, 11 AM to 3 PM, and 4–9 PM are, respectively, 0.308 ± 0.0158, 0.389 ± 0.01935, and 0.322 ± 0.0166. The difference between the first and second is 0.081 ± 0.0250 ($t = 3.24$; $N = 394$), and between the second and third 0.068 ± 0.0255 ($t = 2.64$; $N = 391$). As would be expected were this daily rhythm of dispersion a consequence of a mirror-imaging response to the two magnetic fields, differences and their significance can be substantially increased by taking samples for shorter periods symmetrically arranged over the daily period. For example, selecting the three periods, 5–7 AM, 11 AM–1 PM, and 5–7 PM, one obtains values for standard deviations as follows: 0.276 ± 0.0263, 0.413 ± 0.0272, and 0.276 ± 0.0221. Differences between first and second and second and third are, respectively, 0.137 ± 0.0379 ($t = 3.62$; $N = 173$) and 0.137 ± 0.0351 ($t = 3.91$; $N = 196$), differences which are statistically significant beyond all reasonable doubt ($P < 0.001$).

Hence, it is quite evident that the two magnetic fields are being distinguished by the snails, maximally so over the noon hour, and gradually decreasingly so during approximately the preceding and succeeding six-hour periods.

A lunar-day fluctuation in the difference between the dispersion effected by the two positions of the magnet is also evident. This dispersion is indicated here as standard deviation. Using only the 30-day period, July 6-August 4, when the daily series were the most complete and therefore the solar-daily cycle most fully randomized, the relationship between hour of the lunar-day and the magnetic effect expressed as difference from simultaneous control is shown in Figure 3A. The relative effectiveness of the two magnet orientations is plotted in Figure 3B. In view of the differing sizes of the samples for the various lunar-day hours and days of synodic month, these values were calculated as grouped data for overlapping three-hour periods of the lunar day. The results suggest strongly that the E-W field is relatively the more effective during the hours the moon is below the horizon, and the N-S field during the hours the moon is above the horizon. The N-S field is relatively more effective in inducing dispersion just after lunar zenith, becoming progressively less so both as one proceeds to earlier or later hours of the lunar-day. The E-W magnetic field is most effective just after lunar nadir. There is a suggestion of a brief period of greater effect of the N-S field just preceding lunar nadir. This lunar-day cycle displays a qualitatively comparable relationship of the relative effects of the two fields to that seen for the the corresponding hours of the solar day (see Figure 1B).

A very high statistical significance for the lunar-day cycle of relative effectiveness of the two magnetic fields was demonstrated by finding a coefficient of correlation of 0.752 ($N = 20$; $P < 0.001$) between the difference between the two fields and the hour angle of the moon expressed as deviations from $-22.5°$, through a total of ± 11 hours. For this correlation, the individual lunar-day hourly values, of course, were used rather than the three-hour moving means.

However, during a four-hour period of the lunar-day represented by too few experimental series, the data were combined into two two-hour periods, hence the N of 20 instead of the expected 22.

Using data grouped for three-hour periods it was found that there was a complex lunar-day periodism in the difference between the effectiveness of the two magnet orientations in determining mean snail path. This comprised maximum action by the E-W field in causing left-turning one to two hours after lunar-nadir, and maximum left-turning effect of the N-S field between the times of moon-set and nadir (Fig. 4). Superimposed on this last cycle was a lunar-tidal cycle in which a conspicuous, but secondary, increased effectiveness of the E-W

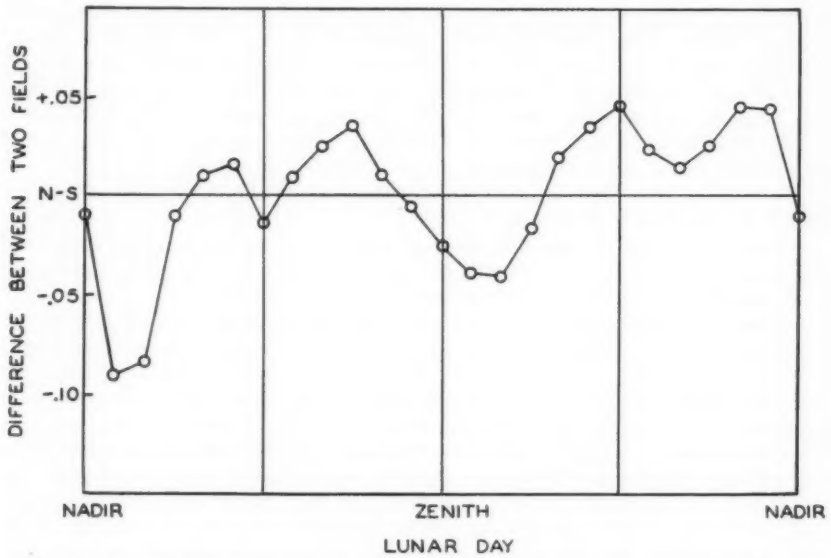

FIGURE 4. The drift through the lunar-day of the effectiveness of the experimental E-W relative to the N-S field in altering mean path from the control one. Points are calculated from three-hour grouped data.

field occurs one to two hours after lunar zenith. There is also a suggestion that this bimodal lunar-day, or lunar-tidal, fluctuation in the relative effects of the two magnetic fields possesses still a further, and lesser, harmonic to give a quadri-modal lunar-day cycle.

It was shown earlier (Brown, Webb and Brett, 1960) that there was, during the two-month period of study, a semi-monthly cycle of direction and degree of turning of the snails induced by the magnets with maximum clockwise turning a day or two before new and full moon, and maximum counterclockwise turning just before the times of first and third quarters of the moon. When now the actions of the two experimental magnetic fields were compared for their contribution to this semi-monthly rhythm, it was found that the two magnetic orientations

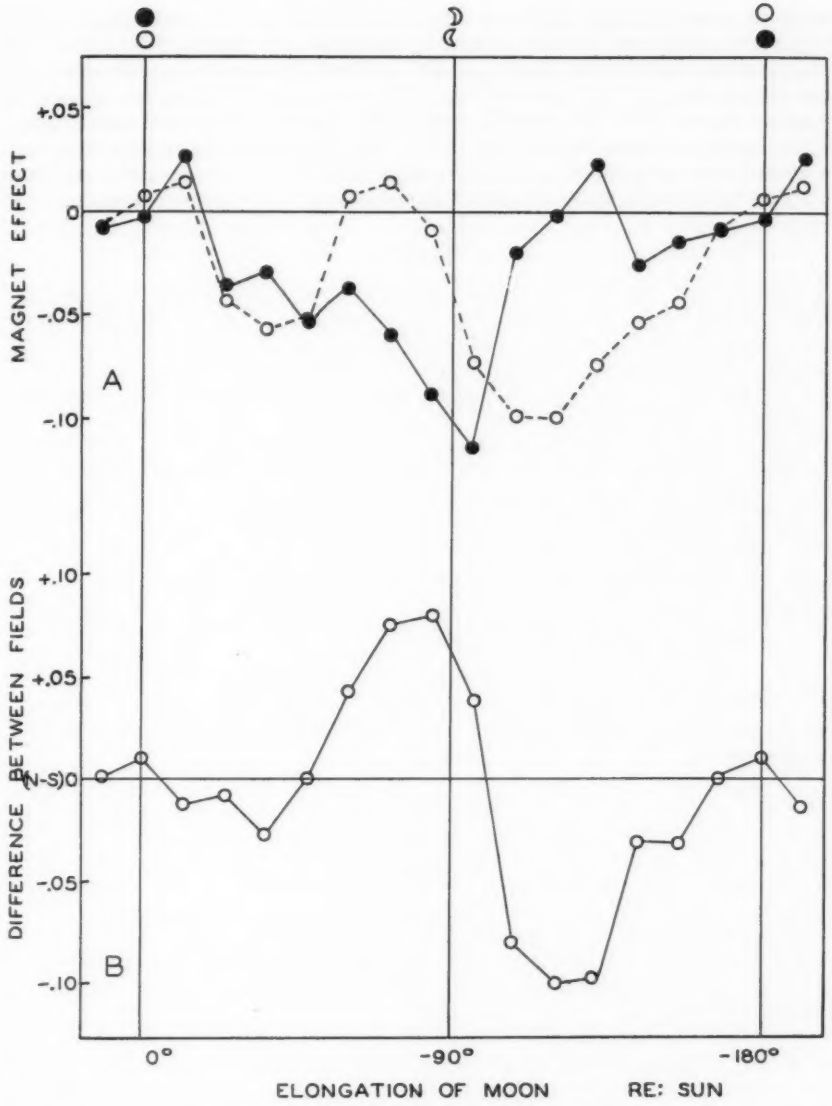

FIGURE 5. A. The drift through the semi-monthly period of the difference between the mean path of snails in the E-W (dashed line) and N-S (solid line) fields from that of the control snails. B. The drift through the semi-monthly period of the effect of the E-W magnetic field relative to the N-S one, in influence on mean snail path. Points are calculated from three-day grouped data.

produced quite strikingly different semi-monthly patterns of effectiveness (Fig. 5A). The difference between them is illustrated in Figure 5B. The E-W field produced relatively a much greater counterclockwise turning about two to four days before the lunar quarters, and then there occurred a very rapid reversal resulting in the N-S field having comparably greater counterclockwise turning action immediately after the lunar quarters. These semi-monthly relationships were computed as overlapping three-day groupings of all data to compensate for greatly differing numbers of series representing the various semi-monthly days.

Discussion

It is evident that the two magnet positions, one with lines of force at right angles to the other, while appearing very similar in effect in indiscriminately pooled data, are in fact not equivalent for the snails at all times of solar and lunar periods. This has been established by the demonstration that the relative effectiveness of the two in inducing left-turning alone, and in affecting total dispersion of pathways (both left and right) exhibit systematic fluctuations through the solar-day, lunar-day and synodic month. In the solar-day, the two fields become essentially equivalent about 8–9 AM and 8–9 PM with difference between the two, in general, increasing systematically during the hours involved in this study as one moves away from these times into the intervening daily hours. For the solar daily cycle, snails moving toward magnetic south in the early morning hours are more strongly turned eastward in an increased magnetic field of E-W orientation than in one oriented 90° to it. On the other hand, by noon, it is the N-S oriented magnet which turns them more strongly eastward. Comparably, in total turning, both eastward and westward, it is the E-W oriented field which produces most turning in the early morning and the N-S field which is most effective from noon to late afternoon. In the daily cycle of response, the snails behave as if they possessed a horizontal directional magnetic axis detector turning through the day like an "antenna."

When the data were rearranged to become lined up in terms of hours of a lunar-day, a quite similar systematic fluctuation was seen, now with the E-W magnetic field exerting the greater turning, either east or west, when the moon was below the horizon, and the N-S field, during the time the moon was above the horizon. This relationship is essentially comparable to the corresponding solar-day one, but the particular phases appear to occur slightly earlier. In this lunar-day fluctuation of the relative influence of the two fields, where all hours of the lunar-day are represented, there is a slight suggestion of a bimodality of the lunar daily cycle with a second, very brief, period of greater effect of the N-S magnet occurring just before lunar nadir.

Although the semi-monthly cycle of the relative effects of the two fields may be at least in some measure an artifact which is a consequence of exclusively daytime sampling, it does lend striking confirmation to the solar-day and lunar-day studies in demonstrating the physiological non-equivalence of the two magnet positions as a function of time.

There seems too little information available to formulate any hypothesis as to how this capacity for magnetic response, so clearly tied in with the fundamental "clock system," might normally operate in orientation of the snail. Orientation

in snail populations may occur to some extent by a magnetoklinokinesis, in which the snails would tend on the average to assume a specific orientation at particular times of day as a consequence of different turning responses for different magnetic orientations, relative to the body axis. However, the clear demonstration that snails can also respond to fields by predominantly either left or right turning, again depending upon time, and can differentiate between two magnetic fields, leaves open the possibility that under some conditions, or at some times of their lives, behavioral response of the snails may possess less the character of a klinokinesis and, instead, more that of a clock-regulated taxis, or even a straightforward compass reaction. The demonstration that in one species, there is a clear clock-regulated capacity to orient in a weak magnetic field and to distinguish directions of magnetic lines of force encourages one to postulate that this fundamental capacity may have become associated with highly evolved and specialized mechanisms in species with unusual homing or other navigational capacities.

In the solar-day and lunar-day cycles of difference between the response to the E-W and N-S fields, the snails are behaving quite as if in their response to magnetic field they were to a small but significant extent slaves of magnetic compass needles within their bodies, with these individual compass needles in turn being hands on two kinds of horizontally oriented clocks, solar-day and lunar-day. Some modification of such a compass response might be expected were the well-known "sun-compass" and "moon-compass" orientations of organisms, with their continuous correction for rotation of the earth, dependent in any way upon a continuously altering relationship of the position of these heavenly bodies relative to the direction of the earth's magnetic lines of flux. This might constitute the regulator of the gradually altering sun-compass or moon-compass angle of orientation of the organisms.

It is, of course, common knowledge that to localize the position of the sun or moon which for one or another reason is not immediately visible, it is not sufficient to have only an accurate record of time. One needs an additional parameter, one indicating spatial orientation on the earth's surface. This could not be better nor more directly provided for than through a general organismic capacity for distinguishing direction in terms of the natural magnetic lines. The possession of "living compasses" along with "living clocks" could therefore constitute a potential means for organismic navigation in the absence of more obvious cues.

This demonstrated capacity for differentiating between the directions of the two experimental magnetic fields, since it deals with a weak field strength relatively close to that of the earth's natural one, reinforces still further the earlier conclusions (Brown, Brett, Bennett and Barnwell, 1960; Brown, Webb and Brett, 1960) that the observed solar and lunar daily fluctuations in orientations by the snails in the earth's natural magnetic field and otherwise in a field symmetrical with respect to all previously recognized orienting factors, are at least in part a true orientation in response to that magnetic field.

Summary

1. The snail is able to differentiate between two weak magnetic fields, one oriented at right angles to the other.

2. The relative influence of experimental north-south and east-west oriented magnetic fields in producing dispersion of snail pathways, or effecting a magneto-klinokinesis, displays both solar- and lunar-day rhythms.

3. The relative influences of experimental N-S and E-W fields in effecting a predominantly counterclockwise snail-turning exhibits solor daily, lunar daily, and semi-monthly rhythms.

4. The solar and lunar clock-regulated discriminatory responses for magnetic fields indicate the snail to be significantly oriented as if by internal magnetic compass needles which in turn are hands of horizontal solar- and lunar-day "clocks."

5. Further arguments are advanced for concluding that organismic orientation to the earth's natural weak magnetic field is a normal organismic phenomenon.

6. Implications of this demonstrated solar and lunar "clock-compass" capacity for the well-known "sun-compass" and "moon-compass" orientations of animals are discussed. Also, the insurance value of a "clock-compass" capacity as a potential navigational system for animals when deprived of celestial references, is pointed out.

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THE OCCURRENCE OF β-ALANINE AND β-AMINOISOBUTYRIC ACID IN FLATWORMS

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β-Alanine and β-aminoisobutyric acid were first shown to be products of uracil and thymine metabolism, respectively, in the rat by Fink and co-workers (1956). Recently, the pathway for the formation of these β-amino acids from pyrimidines has been implicated as part of a celluar homeostatic mechanism which is involved in the regulation of ribonucleic and deoxyribonucleic acid synthesis (Canellakis *et al.*, 1959). Since β-alanine and β-aminoisobutyric acid were found to be prominent free amino acids in three species of cestodes (Campbell, 1960a) and have subsequently been shown to arise metabolically from pyrimidines *via* the pathway elucidated in the rat in a fourth species (Campbell, 1960b), the study of the distribution of these two compounds in flatworms was extended to include other symbiotic and free-living species.

β-Alanine, a product of uracil metabolism, was first reported as a decarboxylation product of aspartic acid in bacteria by Virtanen and Laine (1937). It has since been reported to occur in the free state in certain mammalian tissues (Tallen *et al.*, 1954; Roberts *et al.*, 1950), insects (Clark and Ball, 1951; PoChedley, 1956), various plant tissues (Hulme and Arthington, 1950; Synge, 1951), and recently in several species of mollusks (Simpson *et al.*, 1959). β-Aminoisobutyric acid, a product of thymine metabolism, was first isolated from human urine by Crumpler *et al.* (1951). It has since been reported in tissues of the cat (Tallen *et al.*, 1954), the iris plant (Asen *et al.*, 1959), and the mollusk, *Mytilus edulis* (Awapara and Allen, 1959).

Materials and Methods

The preparation and extraction of the cestodes was carried out as previously described (Campbell, 1960a). The free-living species and the symbiotic species which have a digestive tract were either kept in the laboratory for a sufficient length of time for the tract to empty normally or this was hastened by applying pressure to the organisms with a glass rod. They were then homogenized and extracted by the method used for the cestodes. Total nitrogen in the extracts was determined by a micro-Kjeldahl method (Lang, 1958). The final solution of the free amino acids was made up to contain one mg. nitrogen per ml. Fifty to 300 microliters of this extract were then spotted on Whatman No. 52 paper for chromatography. Two-dimensional chromatography was carried out by the method of Levy and Chung (1953) with the substitution of a sec-butyl alcohol : formic acid : water sol-

[1] A portion of this work was carried out while the author was a Research Fellow of the National Academy of Sciences-National Research Council and was supported in part by U. S. Public Health Service Grant E-1384.

vent system (Hausmann, 1952) in the first dimension. The papers were developed two times in the first dimension. The lowest limit of detection for these two compounds is approximately 0.05 micromoles; consequently, a concentration of β-alanine or β-aminoisobutyric acid in the extracts of less than 0.05 micromoles per 300 microliters extract would not be detected. After the initial chromatography indicated that β-alanine and/or β-aminoisobutyric acid were present, the following methods were used to obtain chromatographic evidence for the identity of these two compounds. In most cases the free amino acid extracts of the flatworms which contained β-alanine and/or β-aminoisobutyric acid were further separated into a basic, neutral, and acidic fraction with Amberlite CG-50 H^+ and Dowex 50 H^+ (Simpson *et al.*, 1959). The neutral fraction contained the β-amino acids. Known β-alanine and β-aminoisobutyric acid added to this fraction or to the extracts gave no additional spots upon two-dimensional chromatography and there was an intensification of the unknown spots in the extracts which were thought to be β-alanine and β-aminoisobutyric acid. The β-amino acids were then separated

TABLE I

R_f values of known β-alanine and β-aminoisobutyric acid and compounds isolated from flatworms

Solvent system	R_f β-Alanine	R_f β-Aminoisobutyric acid
(1) sec-butyl alcohol:formic acid:water (75:15:10, by vol.; developed 2x)	0.52	0.67
(2) m-cresol:phenol:borate buffer, pH 8.3 (30:15:7.5, w/w/v)	0.41	0.53
(3) phenol:water (72:28, v/v)	0.66	0.76
(4) lutidine:water (62:38, v/v)	0.30	0.35
(5) methyl alcohol:pyridine:water (80:4:20, by vol.)	0.49	0.58

Whatman No. 52 paper (solvents 1 and 2) and Whatman No. 3 MM paper (solvents 3, 4 and 5)

from the remainder of the amino acids by streaking the neutral fraction or the extracts on Whatman No. 3 MM paper, chromatographing the papers one-dimensionally in sec-butyl alcohol: formic acid: water two times, and eluting the appropriate portion of the papers to recover the β-amino acids. These eluates, which contained a single β-amino acid, were then concentrated and run in several solvent systems with and without added known β-alanine and β-aminoisobutyric acid. These known compounds were also run as reference compounds by themselves. Identical R_f values were obtained with five solvent systems for both the known compounds and the compounds isolated from the worms. These data are given in Table I. Additional evidence for the nature of the compounds was obtained by pre-treatment of the paper with alkaline copper carbonate. This treatment did not hinder the movement of these compounds during chromatography. Crumpler and Dent (1949) have shown the α-amino acids are rendered immobile as far as chromatography is concerned by the formation of a copper complex, whereas the R_f values of β-amino acids remain relatively the same in the presence of copper.

Results and Discussion

The results of this survey are presented in Table II. β-Alanine and β-aminoisobutyric acid have been identified as free amino acids in all the species of cestodes thus far examined. In *Hymenolepis diminuta*, the concentrations of β-alanine and β-aminoisobutyric acid are approximately equal, amounting to 0.60 ± 0.04 and 0.65 ± 0.08 micromoles, respectively, per gram of tissue (determined by the method of Fowden, 1951). On the basis of the relative intensities of the ninhydrin reaction, β-alanine occurs in higher concentration than β-aminoisobutyric acid in the other four species of cestodes. In *Macrapis cristata*, β-aminoisobutyric acid is in higher concentration than β-alanine. In *Bdelloura candida* and *Entobdella*

Table II

The distribution of β-alanine and β-aminoisobutyric acid in flatworms

Species	Habitat	β-Alanine	β-Aminoisobutyric acid
Turbellaria:			
Bipalium kewense	Free-living (land)	−*	−
Dugesia tigrina	Free-living (fresh-water)	−	−
*Stylochus zebra***	Symbiotic with hermit crab (*Pagurus*)	−	−
Bdelloura candida	Symbiotic upon horseshoe crab (*Limulus*)	+*	+
Trematoda:			
Fasciola hepatica	Endoparasitic in liver of cattle	−	−
*Fascioloides magna***	Endoparasitic in liver of cattle	+	−
Macraspis cristata	Endoparasitic in liver of sting ray (*Dasyatis centrura*)	+	++
Entobdella bumpusi	Symbiotic upon sting ray	+	+
Cestoda:			
Hymenolepis diminuta	Endoparasitic in gut of rat	+	+
Calliobothrium verticillatum	Endoparasitic in gut of dogfish shark (*Mustelus canis*)	++	+
Lacistorhynchus tenuis	Endoparasitic in gut of dogfish shark	++	+
Phyllobothrium foliatum	Endoparasitic in gut of sting ray	++	+
Disculiceps pileatum	Endoparasitic in gut of dusky shark (*Carcharhinus obscurus*)	++	+

* + denotes that the compound was present; − denotes that it could not be detected; ++ denotes that this compound occurs in higher concentration.

** Only one specimen available for analysis.

bumpusi, the two are approximately equal. In this survey only two strictly free-living species were examined, consequently no generalizations can be made concerning the occurrence of these two compounds in symbionts *vs.* free-living species. However, these data indicate that β-alanine and β-aminoisobutyric acid are quite common free amino acids in symbionts which are in contact with host tissue. A notable exception in this case is the liver fluke, *Fasciola hepatica*, which does not have either β-amino acid. It has been shown (Campbell, 1960b) that *H. diminuta* degrades uracil and thymine to β-alanine and β-aminoisobutyric acid, respectively, *via* the intermediate dihydropyrimidines and carbamoyl-β-amino acids which were first shown to be intermediates in rat liver (Fink *et al.*, 1956). Since this path-

way may also be present in the other species of flatworms which have these β-amino acids, the presence or absence of these compounds may reflect the availability of pyrimidines or pyrimidine-containing compounds in the environments of the worms. Another possibility is that the species which do not contain β-amino acids as free compounds are incapable of degrading uracil and thymine. This latter possibility is not known at the present.

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Summary

A survey of thirteen species of free-living and symbiotic flatworms for β-amino acids has been carried out. β-alanine and β-aminoisobutyric acid were found to occur most commonly as free amino acids in the symbiotic species in contact with the tissues of their host. These compounds were not detected as free amino acids in the two free-living species which were examined.

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THE FEEDING MECHANISM IN THE SAND DOLLAR MELLITA SEXIESPERFORATA (LESKE)

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MacGinitie and MacGinitie (1949) give the following account of feeding in the scutellid echinoderm *Dendraster excentricus* (Eschscholtz) (p. 237): "The spines on the upper side of the sand dollar are club shaped and are covered by cilia. These cilia create currents that flow from the direction in which the animal is moving toward what could be called the posterior edge. . . . As the currents flow through these spines, little eddies are created at the posterior sides of the spines. These eddies allow tiny particles and organisms to become trapped in mucus that is secreted onto the surface of the spines. This mucus goes downward and is led into tiny tracts to unite with others. These in turn unite again, passing around the edge to the underside, until near the mouth five tracts or strings of mucus feed directly into the mouth of the sand dollar." This appears to be the only available account of feeding in the Clypeasteroida. Hyman (1958) studied the five-lunuled sand dollar *Mellita quinquiesperforata* (Leske), but was unable to elucidate the feeding mechanism. The present paper describes observations made on another of the lunuled sand dollars, *Mellita* (*Leodia*) *sexiesperforata* (Leske), and shows that it is a ciliary mucus feeder collecting particles on the aboral surface and transporting them through the lunules and around the margin of the test to food tracts on the oral surface.

M. sexiesperforata is common in certain shallow water sandy areas around Jamaica and normally lives either in the surface layer of the sand, so that its outline is discernible from above, or else buried in the sand very close to the surface. The animals used for these observations were collected on the Port Royal Cays and observations were made both in the aquarium and in dishes under a microscope.

Morphology

Figure 1 shows an individual in both oral and aboral views. The size of the animals varies considerably, fully grown animals being about 70 to 80 mm. in diameter. In surface view the animal has a roughly pentagonal outline and its surface is pierced by six slit-like lunules: five of these are in the ambulacral areas, the sixth or anal lunule is interambulacral and marks the posterior side of the animal. The anterior side of the test is markedly pointed. Both aboral and oral surfaces are densely clothed with short spines which are described below. On the aboral side of the only other structures visible are lunules, petaloids, and gonopores. On the oral surface the mouth is central and the anus lies posterior to it, just on the edge of the anal lunule. Leading away from the oral margins of the five ambulacral lunules are a number of broad *food tracts*: one of these, the radial tract, leads straight from the inner tip of the lunule to the mouth, the remainder

run laterally from the lunule and terminate in the *ambulacral* or *food grooves*. These are deep but narrow grooves, a pair to each ambulacral area running into the mouth; the two members of a pair form a petaloid outline and unite just before reaching the mouth. The food tracts of the anal lunule are less well developed and discharge laterally to the ambulacral grooves of the two neighboring lunules.

Figure 2 shows the profile of a sagittal section through the test and shows that it is thin around the margin and dome-shaped in the center; the anterior margin is thicker than the posterior margin. The mean measurements for the ten individuals of 60 to 65 mm. diameter are:

Anterior margin:	1.8 mm.	±0.04
Central dome:	5.9 mm.	±0.49
Posterior margin:	1.0 mm.	±0.01

It is significant that the animal progresses through the sand with the anterior and thicker margin foremost.

Podia

On the oral surface there are dense concentrations of podia around the periphery of the test, around the margins of the food tracts and in the lunules. They are less dense on the remainder of the ambulacral areas and are absent from areas

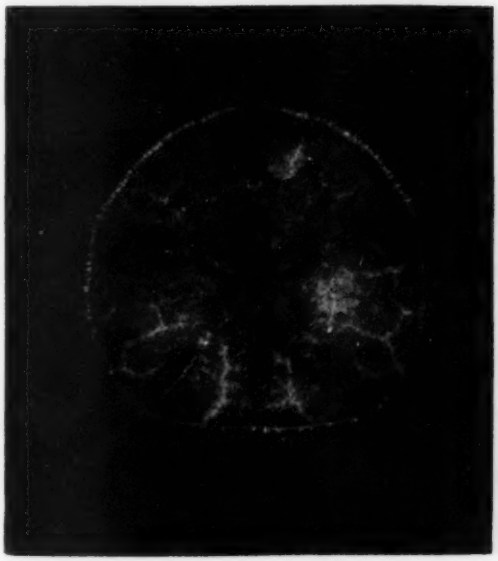

FIGURE 1, a. The aboral surface of a living *Mellita sexiesperforata*. The anterior end is towards the top of the picture. The club-shaped spines appear as minute spots all over the surface of the animal. Notice that the peripheral (ambulatory) spines are more dense anteriorly than posteriorly. The ring of protective spines can be seen clearly around the margin of each lunule.

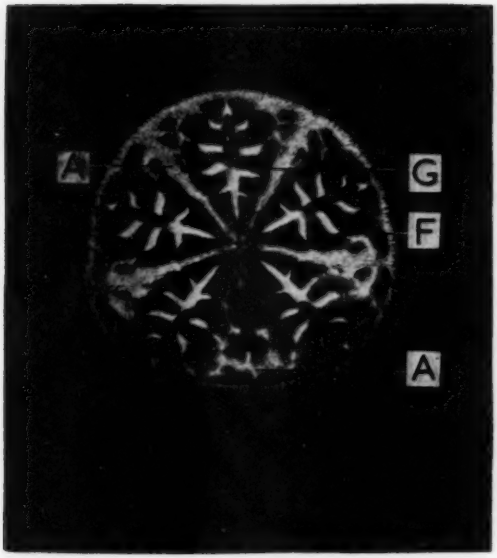

FIGURE 1, b. The oral surface of a living *Mellita sexiesperforata* stained for two minutes in toluidine blue. Orientation as in Figure 1, a. The stain is taken up by podia and other structures, and was used to improve contrast. The mouth is central and the anal papilla appears as a black spot between it and the anal lunule. The anal spines between anus and mouth are just visible. A = Ambulatory spine; F = Food tracts; G = Food (ambulacral) grooves.

with ambulatory spines. On the aboral surface there are only a few scattered podia. When extended the podia are very long and thin with a slightly swollen tip and poorly developed sucking disc. They function exclusively as an accessory food collecting device (see below) and do not appear to play any part in locomotion.

Spines

There are four principal types of spine. On the oral surface ambulatory and non-ambulatory spines can be distinguished. Ambulatory spines are confined to five locomotory areas in the interambulacra of the oral surface. In the anal interambulacrum they form a transverse group behind the anal lunule, in the other four interambulacra they form a radially disposed wedge-shaped group (Fig. 1b). These spines, which are long and thick ($1300\,\mu \times 85\,\mu$) normally have a rounded tip but in many spines the tip is abraded into a roughened end (Fig. 3, A); their

FIGURE 2. Profile of *Mellita sexiesperforata*. The anterior end is toward the right of the picture.

function appears to be entirely that of locomotion. Similar spines are found all around the margin of the test but they are almost twice as thick (1300 μ × 160 μ); they are densely arranged anteriorly and more scattered posteriorly. Their extra thickness is probably correlated with stresses set up during movement through the sand in a horizontal direction. Non-ambulatory or protective spines of the oral surface are shorter and thinner (430 μ × 30 μ) than ambulatory ones and are often bent near the middle of their length (Fig. 3, B); they cover all the remaining areas of the oral surface. Similar spines are found in the lunule and on the aboral margin of the lunule they form a protective ring projecting up higher than the other spines of the aboral surface; here they prevent very large sand particles from entering the lunule and blocking it.

On the aboral surface there are two types of spine distributed together all over the animal. The larger of these are club-shaped spines which move the

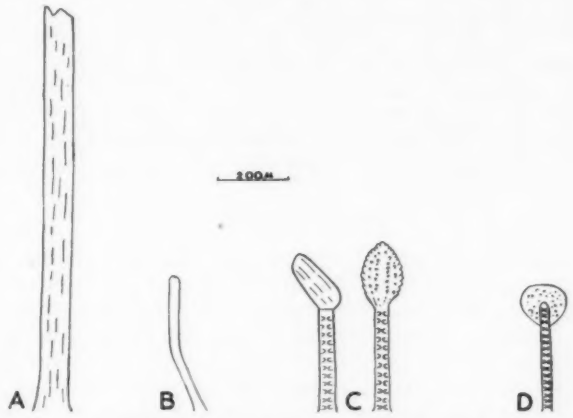

FIGURE 3. The principal types of spine found in *Mellita sexiesperforata*. A = Ambulatory spine with abraded tip. B = protective spine of oral surface. C = Two views of club-shaped spines of aboral surface. D = Aboral miliary spine.

sand grains posteriorly over the aboral surface (Fig. 3, C). The club-shaped head of these spines is set at a slight angle to the stem and its tip is oriented toward the margin of the test. The others or miliary spines (Fig. 3, D) are shorter than the club-shaped spines and are characterized by having a sac-like swelling on the tip; this sac contains yellowish granules which stain darkly with toluidine blue. The epithelium at the base of the cilia on these spines stains purple with the same stain and it seems plausible therefore to suggest that these spines are the principal site of mucus secretion. Miliary spines also occur on the inner walls of the lunules and along the food tracts of the oral surface; the latter have a smaller sac than those of the aboral surface or lunule.

As well as these four main types of spine there is a group of very large spines forming a circle between the mouth and anus and with their tips overarching the anus. They play an important part in preventing feces from entering the mouth.

These spines are in three groups: seven or eight very long ones (up to 5 mm. in length) form a group nearest to the anus, outside these there are ten to twelve of intermediate size, then a group of many smaller ones forming an outer ring next to the mouth. (Fig. 1b).

Cilia

Cilia are confined to the epithelia of the spines and are found on only one side of the stem. This side of the stem is here referred to as its back and the cilia beat in such a way as to drive a horizontal current from it in a backward direction. On the aboral surface the club-shaped spines have dense ciliation at the base while the miliary spines have dense ciliation along the whole length of the stem. The club-shaped and miliary spines are oriented in such a way as to produce centrifugal currents across the aboral surface leading away from the center and towards the margins and lunules. On the oral surface the ambulatory spines have a thin ciliation at the base while the non-ambulatory spines have dense ciliation at the base producing a strong current. The ciliation of the miliary spines of the food tracts is similar to aboral miliary spines. At the margins of the oral surface the spines are oriented so as to drive currents in towards the center. In the food tracts they produce currents towards the mouth in the radial tracts and laterally towards the ambulacral grooves in the remainder. Over the rest of the ambulacral areas the currents drive towards the adjacent ambulacral grooves.

Feeding Behavior

M. sexiesperforata is a microphagous feeder. Removal and microscopal examination of the food cord passing along the ambulacral groove shows that most particles are less than 20 μ in diameter and in a high proportion of them are of the order of 1 μ in diameter. Within these size limits there appears to be no selection of different types of food—algal cells, detrital particles and sand grains are all collected. Larger particles are sometimes collected and sand grains which only just fit in the groove have been seen passing along it and entering the mouth.

An active animal ploughs slowly through the surface sand always keeping the anterior end (Loven's Ray III) forward. As it moves it builds up a small wall of sand in front of itself, the sand grains of which constantly fall down on the aboral surface where they are carried slowly backwards on the tips of the club-shaped spines. This movement of sand appears to be oriented posteriorly across the aboral surface without special reference to the lunules, but small sand grains which reach the margins of the lunules are carried down through them; larger particles are carried off the posterier margin of the test. Particles entering the lunule usually do not drop through it, but are lowered slowly down by means of the spines and then deposited back in the substratum; during this time they may be actively probed by podia in the lunule, suggesting that the latter may remove from it minute absorbed particles.

If carmine particles instead of sand are placed on the aboral surface of the animal a further process of selection may be observed. Larger particles are treated exactly as sand and it can also be seen that the very small particles (visible only under the microscope) drop down between the club-shaped spines and are carried away by the ciliary currents around the spines. At this lower level some particles

travel to the margin of the test and then around it to the oral surface, other particles are carried to the lunules and pass down through them and onto the food tracts on the oral surface. By placing carmine particles in the food tracts of an inverted animal it can be shown that the ciliary currents lead to the ambulacral grooves, except in the radial tract where they lead directly to the mouth. Carmine particles placed on the aboral surface of an animal appear in the ambulacral grooves in a few moments. In the ambulacral groove food can be seen to be loosely aggregated in mucus but the precise point at which the mucus is secreted is still in doubt. As pointed out above the miliary spines of the aboral surface are the most probable site of mucus secretion. In the ambulacral groove the food is carried along by podia and not by cilia.

In summary, then, an animal ploughing through the sand pushes sand onto the aboral surface where it is crudely sorted, large particles being carried along on the tips of the club-shaped spines and ultimately deposited back in the sand either through the lunules or off the posterior edge of the test. Fine particles fall down between the bases of the spines where they are carried away by ciliary currents through the lunules to food tracts on the oral surface. From the food tracts cilia carry them to the ambulacral groove thence to the mouth in a mucus aggregation carried along by podia.

There remains the question of food collection by the podia. Because they are confined to the oral surface it is difficult to see them in normal function, but following the method of Nichols (1959) I have examined them in a perspex box with the aid of a prism and a binocular microscope. Under these circumstances the podia at the margin are seen to be constantly extending and contracting and probing the surroundings of the animal. If carmine particles or yeast stained in congo red are pipetted around the margin, particles may sometimes be seen to be picked up by the podia and drawn in to the margin of the animal where they are released and carried inwards by the ciliary currents. At the same time many podia are seen to extend and contract with no visible particles attached. However, only relatively small magnifications ($\times 30$) can be used successfully in examining with the prism and I believe that the principal function of these podia is to probe the surrounding sand for very small particles of food, *i.e.*, particles of about 1 μ diameter. Mention has already been made of the manner in which sand grains are probed by podia as they pass down through the lunules. In the perspex box only a few scattered grains of sand can be included with the animal; otherwise they obscure it from view. The animal thus rests directly on the bottom of the box and only the marginal podia, which extend laterally, can be extended effectively. It seems certain, however, that under normal conditions the podia of the oral surface all probe the sand in the same way, collecting small food particles.

Defecation

In an animal such as *M. sexiesperforata* in which the anus is in close proximity to the mouth, special precautions are required to ensure that feces do not re-enter the mouth. Although many animals have been examined from time to time defecation has only been observed on a very few occasions and it appears that it must be an intermittent and not a continuous process.

Defecation commences with a pumping action of the anal papilla followed by

cessation of feeding activity; passage of the mucus cord in the ambulacral groove stops completely. The spines between the mouth and the anus beat gently away from the mouth and over the anus, and all around this area pedicellariae become intensely active. The tip of the anus is directed towards the anal lunule and feces are ejected in intermittent puffs of loose particles and not in a mucus string. Examination of a defecating animal from below shows that feces are not, as might be expected, carried up through the anal lunule and so removed in water currents. They fall down from the animal and must in normal circumstances be deposited back in the sand in which the sand dollar community is feeding.

Grateful acknowledgment is made to the Nuffield Foundation from whom the author was in receipt of a research grant when these observations were made.

Summary

1. A brief description of *Mellita sexiesperforata* is given and the process of food collection and defecation are described.

2. Sand pushed onto the aboral surface is sorted by club-shaped spines. Fine particles drop down between the spines and are carried round to the oral surface by ciliary currents, thence to the ambulacral grooves. Mucus is probably secreted by the miliary spines on the aboral surface; podia play an accessory role in food gathering.

3. Defecation is an intermittent process and feeding stops while it is in progress. Spines and pedicellariae prevent feces from reaching the mouth.

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CLEAVAGE WITH NUCLEUS INTACT IN SEA URCHIN EGGS

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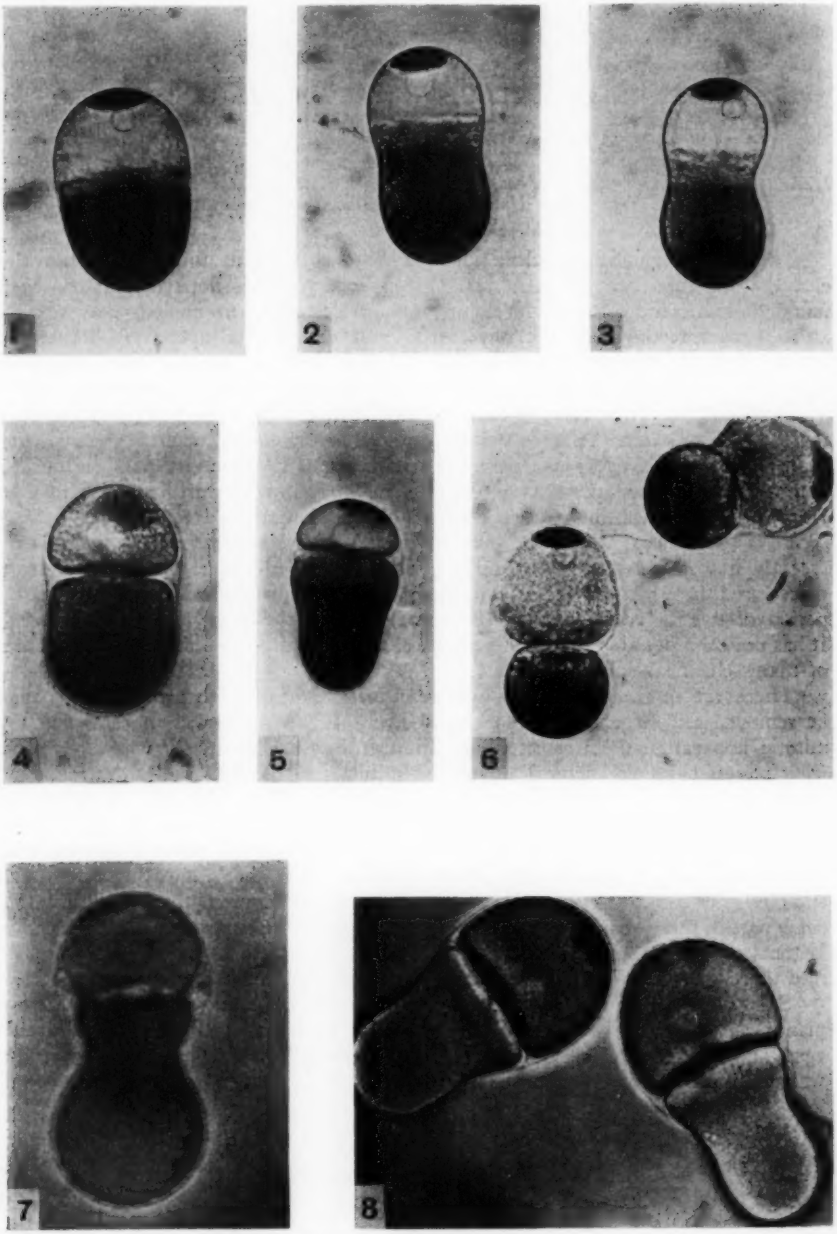
Though, in general, nuclear changes associated with mitosis precede the cleavage of a cell, there are some rare cases in which this is not true. It has been found that in some cases in developing sea urchin eggs, the nucleus may remain as it is in a resting cell, but nevertheless the cell may cleave as it does after mitosis, and produce two quite normal "resting" cells. This has been found to occur in centrifuged eggs which have been stimulated to develop parthenogenetically by treatment with hypertonic sea water (30 grams of NaCl per liter of sea water) for 5 to 15 minutes. After one half to one hour, the egg nucleus remains unchanged but a cleavage plane may come in between the nucleate part of the cell and the non-nucleate part, as it does in such eggs when fertilized (see E. B. Harvey, 1932), resulting in two cells. This has been found to occur in *Sphaerechinus granularis, Psammechinus (Parechinus) microtuberculatus* and more recently in *Arbacia punctulata* and *A. pustulosa* (photographs of living eggs are reproduced in Figs. 1–8). These occasional cases have been observed over a period of twenty years. No further development or change has been observed. It has not been possible to produce such a cleavage lacking nuclear change, with any of many chemical substances tried.

There are in the literature a few references indicating that the nucleus may be removed experimentally. Mazia and Dan (1952) succeeded in removing the mitotic apparatus in an isolated condition from the "fixed" *Strongylocentrotus franciscanus* egg, and later Dan and Nakajima (1956) removed it "fixed" from other sea urchins, *Pseudocentrotus depressus* and *Hemicentrotus pulcherrimus*, with observations also on *Arbacia punctulata*. According to Swann and Mitchison (1953), the eggs of the heart urchin, *Clypeaster japonicus*, may be treated with concentrated colchicine at mid-anaphase, completely abolishing the asters and spindle, and still the egg will divide. There is, of course, the possibility that some part of these structures still remains. To make the experiment more decisive, Hiramoto (1956) sucked out the spindle and asters with a micropipette inserted into the egg, and he found that cleavage still took place.

Some years ago (1938), I made a reference in one of my papers to "cleavage planes coming in independently of any nuclear changes" (p. 182) in sea urchin eggs, and Holtfreter called attention to this in his 1948 paper (p. 723). My paper was accompanied by photographs (44 and 57, 58).

Summary

There now seems no doubt that cleavage can take place without any visible change in the nucleus.



FIGURES 1–8. Living eggs.

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FIGURES 1–3. Stratification of unfertilized eggs of *Arbacia punctulata* with centrifugal force.

FIGURES 4–6. Cleavage of centrifuged, parthenogenetic eggs of *Arbacia punctulata*, without the nucleus taking part. Notice the nucleus in Figure 4, slightly enlarged. It becomes sometimes, not always, slightly enlarged.

FIGURE 7. Cleavage of parthenogenetic, centrifuged eggs of *Sphaerechinus granularis*, without the nucleus taking part.

FIGURE 8. Cleavage of parthenogenetic, centrifuged eggs of *Psammechinus* (*Parechinus*) *microtuberculatus* without the nucleus taking part.

DEVELOPMENTAL STAGES OF THE BROAD BREASTED BRONZE TURKEY EMBRYO[1, 2]

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In studying early mortality in the turkey embryo, it became necessary to determine with considerable accuracy the extent of development of the embryo.

Phillips and Williams (1944) described the Black and the Beltsville Small White turkey embryos after different durations of incubation. However, chronological age, *i.e.*, incubation time, *per se*, is not a reliable expression of the extent of morphological differentiation of the embryo. Such factors as temperature and humidity during incubation, genetic composition, and size of the egg, have been shown to affect the rate of growth of the avian embryo (for review, see Landauer, 1951). It has previously been shown in this laboratory (Kosin and St. Pierre, 1956) that storage of Broad Breasted Bronze hatching eggs for 8 to 14 days results in a lowered mean somite count after 60 hours of incubation, as compared with eggs held for 1 to 7 days.

Hamburger and Hamilton (1951) established a series of normal stages of development for the chick embryo, based on various morphological criteria. These criteria were found to be useful in this laboratory in estimating the extent of development of the turkey embryo, although the turkey embryo takes approximately 28 days to hatch as compared with 21 days in the chicken. Thus, the major objective of the study reported in this paper was to determine for turkey embryos the period of incubation necessary to obtain the different stages of embryonic development described for the chicken by Hamburger and Hamilton (1951).

Similar studies on "staging" of embryonic development in Aves have previously been reported by Rempel and Eastlick (1957) and Koecke (1958) for the White Silkie bantam chicken and the Khaki Campbell and White Indian Runner ducks, respectively.

Materials and Methods

All embryos used in this study were obtained from eggs produced by a flock of Broad Breasted Bronze (BBB) turkeys maintained at the Station. The birds

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[2] Supported, in part, by Federal Funds for Regional Research (W-7) under the Hatch Amended Act.

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[4] We wish to acknowledge the advice and help of Dr. Thomas J. Russell, Washington Agricultural Experiment Stations Statistician, in the analysis of the data on which this study is based. We are also indebted to Mrs. Lynne Frutiger, Mrs. Jewell Keeney, Mrs. Mary Ellen Schy, and Mrs. Jeannette Wright for their technical assistance in the collection of these data.

were trapnested and the eggs were collected three times a day, after which they were placed in the holding room at 50° F. and 85% relative humidity for not more than three to four days. The eggs were incubated for a desired length of time in a forced draft incubator at 99.5° F. The earlier embryos (1 to 7 days) were removed from the yolk, placed in chick Ringer's solution and then measured and staged. Each embryo was staged separately according to (1) the development of the mesodermal derivatives, *e.g.*, somites; (2) the development of the ectodermal derivatives, *e.g.*, optic vesicles, neuromeres; and (3) the development of the heart. The "average" stage of development of the embryo was then obtained from these three separate stagings. Although there were individual differences, no striking and consistent differences between the turkey and the chicken were observed in terms of rate of development in these three groups of morphological criteria. Older embryos (17 to 28 days) were fixed in Bouin's fluid or Baker's calcium formol before staging.

In the later stages of development (Hamburger-Hamilton, stage 36 to stage 40), the turkey embryo has a distinct structure, the "snood" or "leader," which was included among the criteria used for describing the stage of development of the embryo.

Measurements of the beak and toe, which are the main criteria for identifying chick embryos from stage 40 to 44, were obtained for the turkey embryo. However, owing to the relatively small increments of increase in length in these structures, measurements of the foot, *i.e.*, from the outer edge of the tarsal joint to the tip of the claw of the third toe, were used to characterize the development from seventeenth to the twenty-seventh day of incubation.

This study is based on the observation of more than 4000 embryos collected over a period of three years.

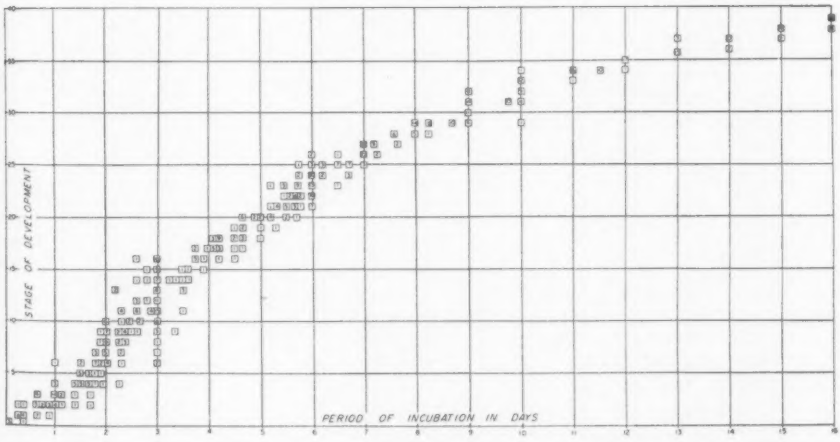

FIGURE 1. Stage (Hamburger and Hamilton, 1951) of development of the turkey embryo after various periods of incubation. The figures in boxes indicate the number of specimens for each point on the coordinate.

TABLE I

Anteroposterior (AP) lengths of the area pellucida and of the BBB turkey embryo at different stages of development

Stage	No. cases	AP length (mm.)	Sd	Embryo length (mm.)	Sd
4	11	3.3	.375	—	—
5	132	3.9	.423	—	—
6	233	4.3	.400	1.4*	.390
7	101	4.7	.458	2.3*	.242
8	52	5.3	.454	3.1**	.341
9	2	5.6	—	3.8	—
10	5	6.3	—	4.6	—
11	9	7.0	.508	5.6	.225
12	12	7.0	.673	5.8	.310

* From the head fold to Hensen's node.
** From the tip of the head to Hensen's node.

RESULTS AND DISCUSSION

The approximate periods of incubation to obtain stages 1 to 39 can be obtained from Figure 1. There is a wide range of variation in the stage of development in the turkey embryo after a definite period of incubation. This becomes particularly apparent in the early stages.

In the earlier stages (stages 4 to 12) the stage of the embryo can also be estimated from measurements of the anteroposterior lengths of the area pellucida or of the embryo, *i.e.*, from the tip of the head, or in stage 6, from the head fold to Henson's node (Table I). These measurements are highly correlated with the stage and somite number (Mun and Kosin, 1958).

The development of the snood ("leader") is summarized in the following tabulation:

Day of incubation	Hamburger & Hamilton Stage	Snood characteristics
13th day	36	Snood appears (Fig. 2).
12 to 14	37	Snood is as high as it is wide at the base (Fig. 3).
15	38	Snood is higher than wide and distinctly columnar in appearance (Fig. 4).
16 to 17	39	Snood is columnar and almost twice as tall as it is wide. Papillae may be seen at the base of the snood (Fig. 5).
17	40	The snood is covered with papillae (Fig. 6).
18	—	The snood is larger and conical feather germs at base of snood are colored (Fig. 7).
19th day	—	The feather germs are as long as the snood and may cover it completely (Fig. 8).

The growth of the beak, toe, and foot from the seventeenth to the twenty-seventh day of incubation is presented, graphically, in Figure 9. Each point represents the average measurements of 10 to 21 embryos by three different individuals on

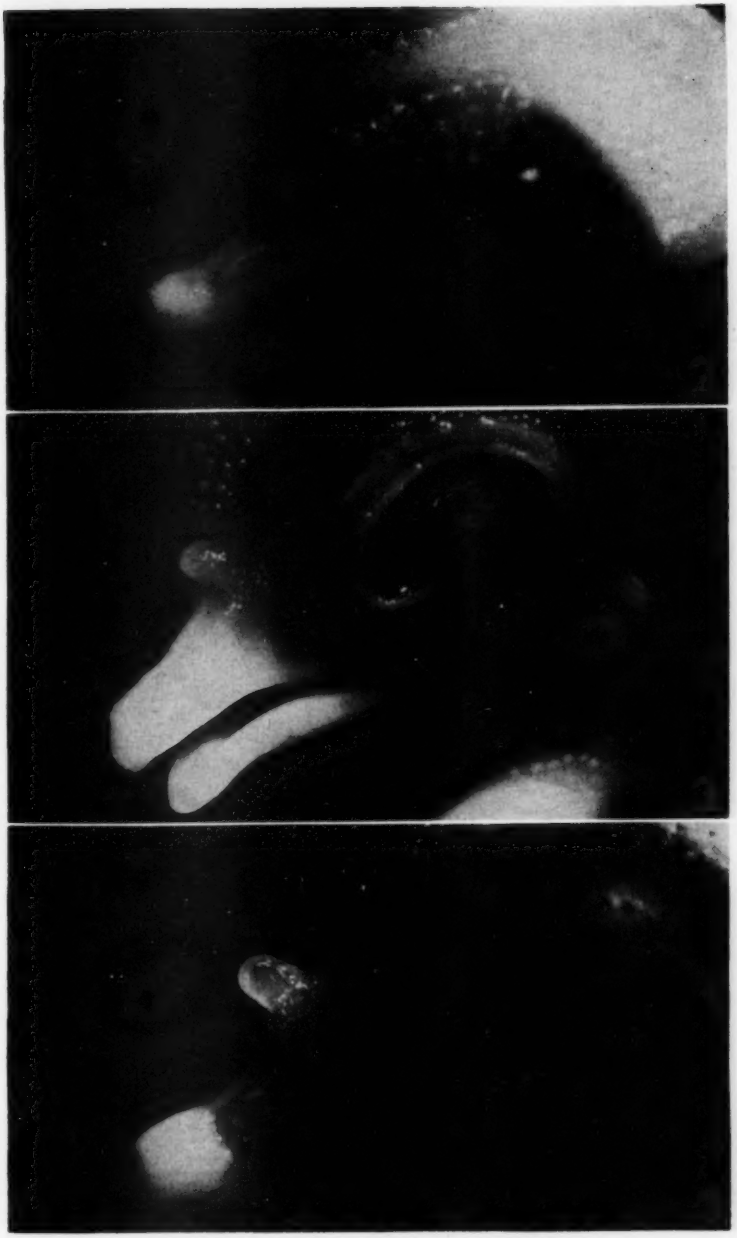

FIGURE 2. Snood at 13th day of incubation.
FIGURE 3. Snood at 14th day of incubation.
FIGURE 4. Snood at 15th day of incubation.

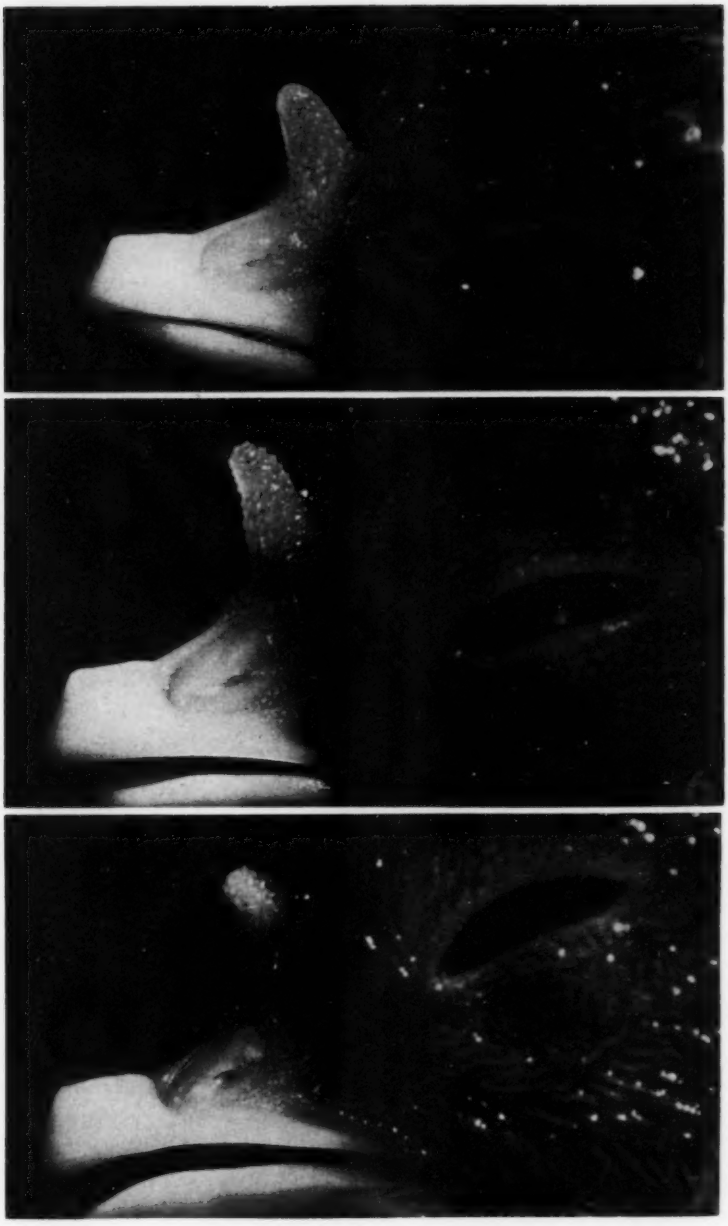

FIGURE 5. Snood at 16th day of incubation.
FIGURE 6. Snood at 17th day of incubation.
FIGURE 7. Snood at 18th day of incubation.

FIGURE 8. Snood at 19th day of incubation.

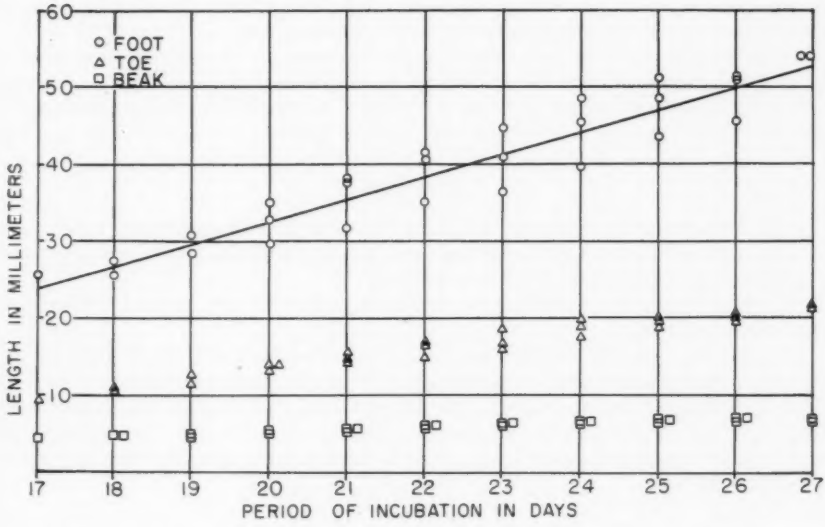

FIGURE 9. Length of beak, toe, and foot after different periods of incubation.

different groups of embryos at different times. The measurements were made with a pair of vernier calipers.

As may be seen from Figure 9, the rate of growth of the foot is greater than the rate of growth of the beak and toe. A straight line obtained from the regression of the length of the foot on time was then constructed. The equation of this line (solid line in Figure 9) is as follows:

$$Y = -25.3 + 2.9x$$

where Y = length of the foot in mm. and x = period of incubation in days.

The standard deviation of the regression line is 3.3 and the standard error of the slope is 0.051.

The 95% confidence interval in age of the embryo, for any particular length of the foot, can be calculated from the following equation:

$$\left.\begin{matrix} \bar{L}x \\ Lx \end{matrix}\right\} = 36.6 + 0.35Y_0 \pm 0.7\sqrt{0.00024(Y_0 - 40.0)^2 + 8.342}$$

where $\bar{L}x$ = upper limit of age of the embryo in days, Lx = lower limit of age of the embryo in days and Y_0 = observed length of the foot in millimeters.

Similarly, the 95% confidence interval for the length of the foot following a definite interval of incubation can be calculated from the following equation:

$$\left.\begin{matrix} \bar{L}y \\ Ly \end{matrix}\right\} = -25.3 + 2.9X_0 \pm 6.47\sqrt{1.002 + 0.00024(X_0 - 22.6)^2}$$

where $\bar{L}y$ = upper limit of the length in mm., Ly = lower limit of the length in mm. and X_0 = observed period of incubation in days.

This information has been used in our laboratory to approximate the time of death of the embryo, whether it was accidental, *e.g.*, due to incubation failures, or due to causes associated with the problem of hatchability, and to compare the growth rates of embryos from different lines of BBB turkeys cultivated *in vitro*.

Summary

1. The period of incubation of Broad Breasted Bronze turkey eggs necessary to obtain the various normal stages of development established by Hamburger and Hamilton for the chick embryo is presented.

2. Data have been submitted on the development of the snood ("leader") in the turkey embryo.

3. Measurements of the beak, toe, and foot were obtained from the seventeenth to the twenty-seventh day of incubation. From these measurements, a straight line obtained from the regression of the length of the foot on time was constructed. The equation of this line is presented, as well as equations for determining the approximate age of the embryo from measurements of the foot, or the approximate length of the foot.

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CAROTENOIDS AND CHLOROPHYLLIC PIGMENTS IN THE MARINE SNAIL, CERITHIDEA CALIFORNICA HALDEMAN, INTERMEDIATE HOST FOR SEVERAL AVIAN TREMATODES [1, 2]

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The marine snail, *Cerithidea californica* Haldeman, is a favorable host for more than twenty species of larval trematodes (Martin, 1955; Hunter, 1942). These larvae occupy different regions of the body of the snail such as the digestive gland, mantle, gills and part of the digestive tract. The digestive gland of the snail, which is the main organ of infection, presents a variety of coloration in different specimens. It may be green, brown, yellow, orange or creamy white. The visceral part including the digestive tract is frequently dark blue. The mantle and the integument are usually greenish-blue or blue and yellow intermingled. A striking similarity exists between the coloration of the snail tissues and that of the parasitic larvae harbored by them.

A considerable amount of information is available concerning the occurrence and distribution of pigments, particularly carotenoids in various species of gastropod molluscs. Earlier work has been reviewed by Fox (1953) and Goodwin (1954). Although several species of snails are known to be hosts for pigmented larval trematodes, no critical study so far has been made concerning their pigments with a view to understanding the host-parasite relationship of pigmentation. In the snail, *Littorina littorea*, pigmented foot has been reported to be a means of recognizing infection with larval trematodes (Willey and Gross, 1957). Spectrophotometric absorption studies of *L. littorea* extracts indicated the presence of carotenoids; however, chromatographic methods were not used for the separation of various pigments. The author was interested to study the chemical nature and origin of pigments found in certain species of larval trematodes harbored by the snail, *Cerithidea* (Nadakal, 1960a, 1960b). In order to trace the host-parasite relationship of pigments, it was necessary to analyze the pigments of the snail. The present paper describes the pigments found in *Cerithidea* with special reference to carotenoids.

Materials and Methods

Specimens of *Cerithidea* and four species of algae, including three green and one red algae which serve as food for the snails, were collected from the mud flats of Newport Bay, California. The green algae were identified as *Ulva* sp., *Chacto-*

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morpha torta, and *Enteromorpha clathrata;* the red alga as *Hypnea johnstonii.* The algal pigments were studied by the methods of the following workers: Strain (1942) for chlorophyll *a* and *b;* Manning and Strain (1943) for chlorophyll *d;* and Haxo *et al.* (1955) for phycobilins. After sorting out the various snail tissues such as the digestive gland, mantle and foot, and visceral mass, they were lyophilized separately and ground in an ordinary mortar. The methods outlined by Fox and Pantin (1941) were followed with necessary modifications for the extraction and analysis of pigments from the snail tissues. Chromatographic separation of pigments was carried out according to the directions given by Karrer and Jucker (1950). A cylindrical glass tube measuring 20 cm. × 12 mm. was used for preparing the adsorption column. Among several adsorbents tried for the separation of various pigments, such as calcium hydroxide for epiphasic carotenoids, calcium carbonate and zinc carbonate for hypophasic carotenoids, and powdered sugar (C & H Confectioner's) for chlorophyll derivatives, activated alumina was found most satisfactory. Various pigment fractions obtained by chromatographic separation were eluted in appropriate solvents like petroleum-ether, methanol, etc. for determining the absorption spectra, with a Beckman Spectrophotometer. Efforts were made to identify the pigments by spectrophotometric absorption analyses, partition tests, color reactions, solubility, fluorescence, and chromatographic behavior.

Figures 1–3 include spectral curves of the pigments A-E in petroleum-ether (b.p. 50–70° C.) and F and G in methanol.

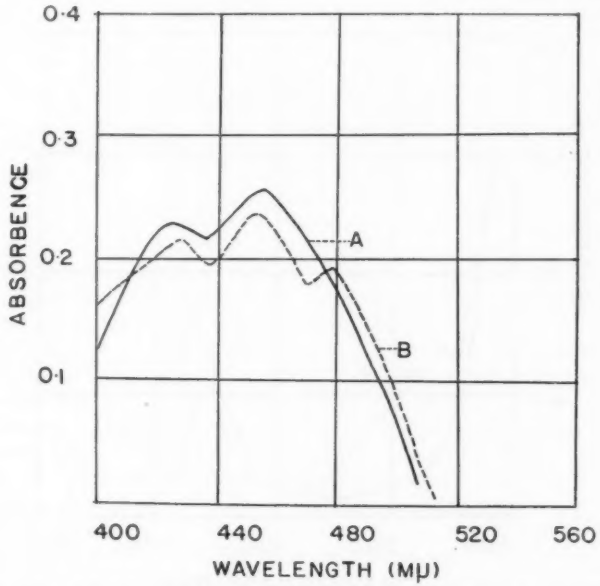

FIGURE 1. A. Light orange pigment HIII (Table II). B. Yellow pigment HII (Table II).

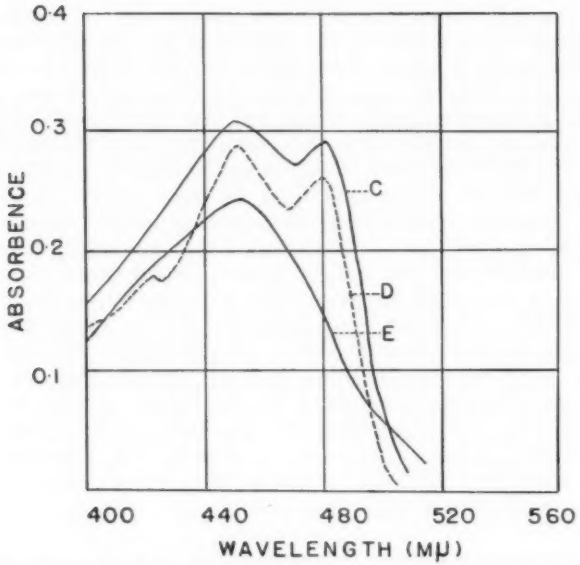

FIGURE 2. C. Orange pigment EIV (Table I). D. Orange pigment EIII (Table III). E. Violet pigment EIII (Table I).

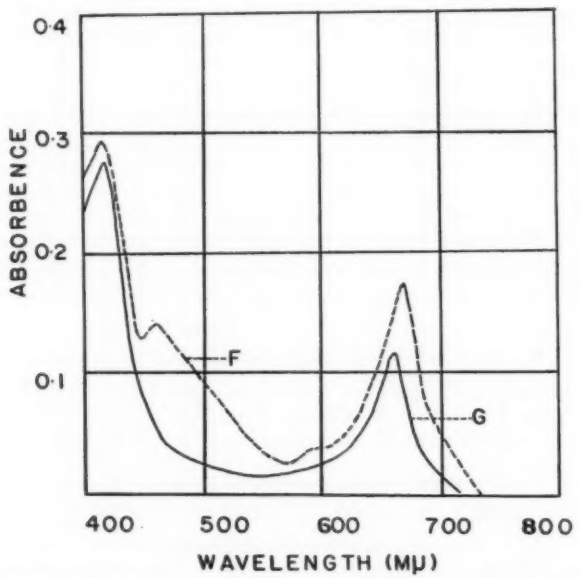

FIGURE 3. F. Yellowish-brown pigment EII (Table I). G. Pale green pigment EIIa (Table I).

Results

The pigment fractions obtained by chromatographic separation of the epiphasic and hypophasic portions of the pigment extracts of the various snail tissues and their characteristics are listed in Tables I-V. The pigment fractions in the case of epiphasic portions of the extracts are numbered in order of decreasing adsorption and hypophasic portions in order of increasing adsorption on the columns.

Absorption maxima and the forms of the spectral curve (Fig. 3, G) of the pigments EI and EIIa (Table I) indicate that these pigments resemble chlorophyll or pigments derived from chlorophyll. However, the absorption maxima in the violet region of the spectrum are different from that of the chlorophyll reported from plant sources. The Gmelin reaction (Pearse, 1953) was negative for these pigments, suggesting that they are not open-ring tetrapyrrole compounds.

The brown pigment EIIb (Table I) showed a maximum at 450 mμ. This fraction could not be made hypophasic even after prolonged saponification. This may be a carotenoid acid.

Table I

Epiphasic portion of the digestive gland extract. Adsorbent: activated alumina. Developing solvent: petroleum ether (50–70° C.) with 1–5% methanol

Band No.	Color of band	Percentage of solvent required for elution	Absorption maxima mμ	Solvent
EI	Greenish-yellow	2–3 methanol	410, 667	Ethanol
EII	Yellowish-brown	2–3 methanol	410, 450, 667	Ethanol
EIII	Violet	3–4 methanol	454	Petroleum ether
EIV	Orange	0	452, 482	Petroleum ether
Band EII was chromatographed again on alumina column and the two fractions obtained are given below:				
EIIa	Pale green	2–3 methanol	416, 665	Methanol
EIIb	Brown	3–4 methanol with a few drops of glacial acetic acid	450	Petroleum ether

The violet pigment EIII (Table I) showed a maximum at 454 mμ. The form of the spectral curve (Fig. 2, E) and the single absorption maximum are suggestive of a keto-carotenoid (Vevers and Millott, 1957).

The orange pigment EIV (Table I) has been found to possess properties similar to those of β-carotene (Fig. 2, C). The absorption maxima are in good agreement with the figures given by Karrer and Jucker (1950), and Lederer (1938). Besides, solubility, behavior on partition test, fluorescence (bluish-green in ultra violet light), color reactions (pigment in chloroform solution turned bluish-green on addition of concentrated sulfuric acid), color in solutions, and chromatographic behavior also indicate that this is β-carotene.

The absorption maxima of the pink pigment HI (Table II) in petroleum ether and benzene are in good agreement with the figures given by Karrer and Jucker (1950), and Goodwin (1953) for zeaxanthin. Its hypophasic behavior on partition test and chromatographic behavior also lend support to the conclusion that

TABLE II

Hypophasic portion of the digestive gland extract. Adsorbent: activated alumina. Developing solvent: petroleum ether with 1–5% methanol

Band No.	Color of band	Percentage of solvent required for elution	Absorption maxima mμ	Solvent
HI	Pink	2–3 ethanol	420, 450, 482 462, 490	Petroleum ether Benzene
HII	Yellow	1–2 methanol	426, 448, 478 429, 457, 487	Petroleum ether Chloroform
HIII	Light orange	1–2 methanol with a few drops of glacial acetic acid	424, 454	Petroleum ether
HIV	Blue-green	2–3 ethanol	416, 670	Methanol

this is zeaxanthin. The fact that this pigment fraction stayed hypophasic even after continued saponification and could not be made epiphasic with acid treatment, showed that this pigment occurs in the snail tissues in the free state.

The yellow pigment HII (Table II) showed maxima in petroleum ether and chloroform which are in good agreement with the figures quoted by Karrer and Jucker (1950) for the xanthophyllic pigment lutein (Fig. 1, B). This pigment turned bluish-green with concentrated sulfuric acid. These properties, coupled with the chromatographic behavior, hypophasic nature on partition test, and color in solutions, prove that this is lutein.

A yellow pigment fraction, separated from the epiphasic portion of the unsaponified pigment extract, showed maxima in petroleum ether at 424, 445, and 475 mμ. This pigment became hypophasic on saponification and could be made epiphasic again with acetic acid treatment. This indicated that some of the lutein in the snail's tissues is esterified. After saponification the pigment showed the maxima at 446 and 478 mμ in petroleum ether.

The light orange pigment HIII (Table II) could be removed from the column with a few drops of acetic acid in the eluting solvent. It is difficult to identify this pigment conclusively; it may be a carotenoid acid or some pigment derived from hypophasic carotenoids (Fig. 1, A).

The blue-green pigment HIV (Table II) is characterized by absorption maxima and spectral curve suggestive of chlorophyll or a pigment derived from it.

The brown pigment EI (Table III) showed only one absorption maximum.

TABLE III

Pigment extracts from the mantle, branchial, and pedal tissues. No hypophasic fraction was obtained on partition of the original extracts between 90% methanol and petroleum ether systems. Pigments chromatographed as the epiphasic portion of the digestive gland extract

Band No.	Color of band	Percentage of solvent required for elution	Absorption maxima mμ	Solvent
EI	Brown	2–3 methanol	450	Petroleum ether
EII	Pink	1–2 methanol with a few drops of glacial acetic acid	452	Petroleum ether
EIII	Orange	0	424, 452, 480	Petroleum ether

This fraction could not be made hypophasic even after prolonged saponification in 90% methanol-petroleum ether systems. Identification of this pigment fraction was difficult.

The pink pigment EII (Table III) could be eluted with a few drops of glacial acetic acid. It showed a maximum at 452 mμ. On saponification this became hypophasic and could be made epiphasic again by treating with acetic acid. This behavior indicated that this pigment could be an esterified carotenoid acid.

The orange pigment EIII (Table III) showed all characteristics of β-carotene. However, a small shoulder at 424 mμ in the spectral curve of this pigment is remarkable (Fig. 2, D).

The khaki pigment EI (Table IV) showed an absorption maximum only in the violet region of the visible spectrum. This may be some breakdown product

TABLE IV

Pigment extract from the visceral mass. Epiphasic portion chromatographed on activated alumina. Developing solvent: petroleum ether with 1–5% methanol

Band No.	Color of band	Percentage of solvent required for elution	Absorption maxima mμ	Solvent
EI	Khaki	1–2 methanol	425	Methanol
EII	Greenish-yellow	2–5 methanol	416, 667	Methanol
EIII	Yellowish-brown	2–4 methanol	420, 452, 667	Methanol
EIV	Violet	2–3 methanol	452	Petroleum ether
EV	Orange	0	450, 482	Petroleum ether
Band EIII was chromatographed again on alumina column and the two fractions obtained are given below:				
EIIIa	Pale green	1–2 methanol	416, 665	Methanol
EIIIb	Pale orange	1–2 methanol with a few drops of glacial acetic acid	450	Petroleum ether

of chlorophyll or carotenoid pigments. Such breakdown products are known to be adsorbed at the top of the columns (Fox, 1953).

The spectral properties of the pigments EII and EIIIa (Table IV) indicated that they are chlorophyll derivatives.

The pale orange pigment EIIIb (Table IV) is considered to be a carotenoid acid because of its single absorption band and acidic properties.

The violet pigment EIV (Table IV) was more or less similar to the violet pigment EIII (Table I) extracted from the digestive gland and may be a keto-carotenoid.

The orange pigment EV (Table IV) was identical with the pigments EIV (Table I) and EIII (Table III) extracted from the digestive gland and mantle, respectively. It is therefore concluded to be β-carotene.

The pink pigment HI (Table V) was similar in properties to the one HI (Table II) recovered from the hypophasic portion of the digestive gland extract and is concluded to be zeaxanthin.

The yellow pigment HII (Table V) has been identified as lutein, as its properties resemble those of the yellow pigment HII (Table II) extracted from the hypophasic portion of the digestive gland extract.

The light orange pigment HIII (Table V) with its acidic properties and two absorption maxima in the blue-violet region of the visible spectrum may be considered as a carotenoid acid. No trace of chlorophyll derivatives could be detected in the hypophasic portion of the visceral extracts.

A blue-green residue was left in the methanol-water fraction of the original extracts of the digestive gland and visceral tissues. Part of the colored substance could be taken up in ether after addition of a few drops of glacial acetic acid. It was then washed with water, evaporated to dryness under vacuum and finally taken up in methanol. The absorption maxima of this pigment at 416 and 667 mμ and the form of the spectral curve was characteristic of chlorophyll *a* (Atkins and Jenkins, 1953; Green, 1957). Even after extraction of the chlorophyllic pigments by acidified ether, a bluish residue was left behind in the aqueous methanolic solution. This was not extractable by any of the solvents tried. Attempts to

TABLE V

Hypophasic portion of the visceral extract. Chromatographed as the hypophasic portion of the digestive gland extract

Band No.	Color of band	Percentage of solvent required for elution	Absorption maxima mμ	Solvent
HI	Pink	2–3 methanol	420, 450, 482	Petroleum ether
HII	Yellow	1–2 methanol	422, 448, 478	Petroleum ether
HIII	Light orange	1–3 methanol with a few drops of glacial acetic acid	424, 454	Petroleum ether

separate the pigment on adsorption columns also failed. This bluish residue probably contained haemocyanin which is common in molluscan body fluids.

Pigments Found in the Algae

The three species of green algae, *Ulva* sp., *Enteromorpha clathrata*, and *Chaetomorpha torta*, have been found to contain chlorophyll *a* and *b*, β-carotene, and the xanthophyllic pigment lutein. No trace of α-carotene or any other pigments related to carotenoids could be detected. The red alga, *Hypnea johnstonii*, contained chlorophyll *a* and *d*, β-carotene, lutein, phycoerythrin, and phycocyanin. The principal carotenoid pigment extracted from the algae was β-carotene.

Discussion

The occurrence of carotenoids and chlorophyll derivatives in *Cerithidea* is in agreement with the previous findings in various species of molluscs. β-Carotene and lutein have been reported from several gastropods (Fox, 1953; Goodwin, 1954). Zeaxanthin is known to occur in *Patella vulgata* and *P. depressa* (Goodwin, 1954; Goodwin and Taha, 1950), and *Mytilus californianus* (Scheer, 1940). Carotenoid acids with single absorption bands in the visible spectrum have also been reported

from many invertebrates such as sponges and molluscs (Fox, 1953). Demonstration of chlorophyll derivatives in molluscs has been made by MacMunn (1886a, 1886b), Dhéré and Vegezzi (1916), and many others.

A survey of the occurrence and distribution of carotenoid pigments in invertebrates reveals the fact that the digestive gland plays an important role in the storage of these pigments. Crane (1949) found that in the cephalopods, *Octopus bimaculatus* and *Loligo opalescens*, the liver-pancreas accumulated relatively large amounts of carotenoid pigments. In *Cerithidea*, undoubtedly the digestive gland functions as the chief organ for the storage of carotenoids. The preferential accumulation of carotenoids in the lipid-rich digestive gland is not surprising since these pigments have a tendency to be associated with lipids.

The visceral extracts of *Cerithidea* have also been found rich in carotenoid and other pigments. This might be due to the presence in the gut of plant materials ingested as food. The mantle and branchial tissues contained relatively small amounts of carotenoids and no traces of chlorophyll derivatives could be detected in these tissues. A similar situation is described by Brooks and Paulais (1939) in the lamellibranchs, *Ostrea edulis* and *Gryphaea angulata*.

In *Cerithidea*, the principal pigment found in various tissues is β-carotene. Next in importance, on a quantitative basis, are the chlorophyll derivatives; third comes the lutein and fourth only the zeaxanthin. Carotenoid acids and ketocarotenoids occur in traces only. The snail apparently shows a preference for storing β-carotene in its tissues. This is perhaps due to the preponderance of β-carotene in the algae which serve as food for the snail. Since *Cerithidea* assimilates and stores both hydrocarbons and xanthophylls as well as chlorophyll derivatives, it may be regarded as non-selective in its chromatic storage. However, the preferential accumulation of carotenes in the mantle and the branchial tissues is remarkable indeed.

There is ample evidence for the elementary origin of carotenoids and chlorophyll derivatives in animals. The crustacean, *Daphnia*, builds up its carotenoid supply from the algae upon which it feeds (Green, 1957). The sea mussel, *Mytilus californianus*, is known to absorb and store carotenoid pigments "from a very plentiful and widely varied diet" (Fox and Coe, 1943). Dhéré and Vegazzi (1916) concluded from experimental evidence that the greenish and grayish hepatic pigments of *Helix pomatia* were derived from the chlorophyll of its diet. The dark green pigment "chaetopterin" found in the intestinal epithelium of the polychaete worm, *Chaetopterus*, is derived from chlorophyll by the elimination of magnesium and the phytol chain (Lederer, 1940). Similarly, Lederer and Huttrer (1942) and Winkler (1957) showed that the sea slug, *Aplysia*, accumulates the pigment "aplysioviolin" in its ink-gland, which is derived from red algae consumed as part of its diet.

As regards *Cerithidea*, sources of pigments could also be attributed to nutritional factors. Examination of four species of algae which serve as food for the snail indicated the presence of chlorophylls and carotenoids, in addition to the phycobilins and chlorophyll *d* in the red alga. The snail probably builds up its pigment supply from these algal sources. β-Carotene seems to have been accumulated in the snail tissues without any metabolic alteration. However, the bluish-green color of mantle tissues is an indication that carotenoids may exist in them as a carotenoid-

protein complex. The fact that no chlorophyll derivatives could be detected from these tissues also lends support to this conclusion. Zeaxanthin, carotenoid acids, and keto-carotenoids were not observed in the algae studied; they may be considered as products of metabolic activities of the snail. Moreover, some of the lutein and carotenoid acids were found esterified in the tissues of the snail. The spectral properties of chlorophyll derivatives indicate that these pigments must have also undergone some kind of metabolic change, possibly oxidation. The absorption maxima of the chlorophyll derivatives in the red region of the spectrum suggest that these pigments are derived from chlorophyll *a* of the algae. There was no indication of the presence of chlorophyll *d* or phycobilins in the tissues of the snail, negating the possibility that the snail absorbs these pigments from the red alga, *Hypnea*.

Several examples can be cited to prove that the accumulation of pigments in the body tissues of animals frequently results from catabolic activities. Such accumulation of pigments may or may not be significant in the functional economy of these organisms. It has been reported that the large pigment cells found in the deeper layer of connective tissues adjoining the intestinal caeca of the leech, *Glossiphonia complanata*, represent a kidney for the storage of waste products derived from haemoglobin metabolism (Bradbury, 1957). Wigglesworth (1943) found that in the blood-sucking bug, *Rhodnius prolixus*, some of the ingested blood is denatured to form biliverdin which is subsequently either excreted through the gut or stored in the pericardial cells. Stephenson (1947) noticed that the pigment in the gut epithelium of *Fasciola hepatica* is derived from the haemolysis of the ingested blood. The occurrence of chlorophyll derivatives in *Cerithidea* may not have any functional significance; they simply happen to be deposited in the tissues as metabolic wastes resulting from the digestion of algal food. Nevertheless, the storage of carotenoids in various tissues, particularly in the digestive gland of the snail, may be beneficial since there are indications that certain carotenoids may serve to prevent autoxidation of lipids in animal tissues (Verne, 1936a, 1936b). It is yet to be found out whether the snail needs vitamin A for its metabolic activities, and, in case it does, it might make use of β-carotene as a potential source. Although in certain invertebrates carotenoids are known to be utilized in sexual reproduction (Scheer, 1940) and in maintenance of mucous surfaces, nothing is known about their roles in similar processes in *Cerithidea*.

Summary

1. Evidences obtained from chromatography, spectrophotometric absorption analyses, partition tests, etc. suggested the occurrence of the following pigments in the marine snail, *Cerithidea californica*: β-carotene, carotenoid acids, keto-carotenoids, lutein, and chlorophyll derivatives.

2. In an attempt to understand the dietary relationship of pigmentation in the snail, four species of algae were studied for their pigment contents. The three green algae were found to contain chlorophyll *a* and *b*, β-carotene, and lutein; the red alga, chlorophyll *a* and *d*, β-carotene, lutein, and phycobilins.

3. The spectral properties of the chlorophyll derivatives recovered from the snail suggested that they are derived from chlorophyll *a* of the algae and that the molecular structure is still intact with the magnesium atom atached to it. However,

absorption maxima in the violet region of the spectrum are shifted toward shorter, and in the red toward longer wave lengths, indicating some metabolic change in these pigments, possibly oxidation.

4. Part of the lutein and carotenoid acids were found to be esterified in the digestive gland and mantle tissues. No metabolic alteration has been noticed in the case of β-carotene. All available evidence suggests that zeaxanthin, carotenoid acids, and keto-carotenoids are products of the snail's metabolic activities.

5. Apparently the snails do not absorb phycobilins or chlorophyll *d* from the red alga.

6. The snail has been found to be non-selective in its chromatic storage.

7. The nutritional relationship and biological significance of pigments in the snail have been discussed.

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THYROID HORMONE TREATMENT AND OXYGEN CONSUMPTION IN EMBRYOS OF THE SPINY DOGFISH [1]

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The inability of adult cold-blooded vertebrates to respond to thyroxine treatment with an increased oxygen consumption rate is now a well documented finding (Hoar, 1957; Gorbman, 1959). The two often cited exceptions to this general experience are the thyroxine-induced increases in oxygen consumption in adult goldfish observed by Müller (1953), and in parrot fish of certain sizes, as described by Smith and Matthews (1948). Both of these claims have been denied by opposite results in the same species (Etkin, Root and Mofshin, 1940; Chavin and Rossmore, 1956; Matty, 1957). Measurements of metabolic rate in fishes are subject to numerous variables which are not as easily controlled as they are in mammals (responses to handling, previous temperature history, illumination, endogenous activity cycles) (Fry, 1957), so that it is not surprising that conflicting claims may exist for some species. Of the factors which may contribute misleading information in measurements of oxygen consumption in fishes, among the most significant is muscular activity. Hoar (1958) has shown clearly that treatment of fishes with thyroid hormone induces behavioral changes, expressed primarily by an increased spontaneous motor activity. If this is so, then any valid test for basal metabolic stimulation by thyroxine must exclude the variable of locomotor muscular work. Although testing systems are available which make this possible (Fry, 1957), neither of the two exceptional claims mentioned above utilized them.

While working with near-term embryos of the spiny dogfish, *Squalus suckleyi*, removed from the uterus and kept in flowing sea water, we noticed a behavioral feature which makes this animal useful in respiratory studies. When kept in subdued light they remain still, even after treatment with thyroid hormone. Since under these circumstances, spontaneous muscular movements are rare, then respiratory measurements can be taken to reflect "basal" requirements (or at least "standard" metabolism as defined by Fry, 1957), not a thyroxine-induced increase in swimming. In these experiments oxygen consumption of such exteriorized dogfish pups was measured after treatment with thyroxine, or two of its analogues, or propyl thiouracil. To our knowledge the only other studies of the metabolic responsiveness to thyroid hormone in larval vertebrates have concerned anuran tadpoles. In this regard, too, the published literature is in disagreement (Etkin, 1955; Lewis and Frieden, 1959). Accordingly, it was hoped that the experiments

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with dogfish embryos would prove enlightening, both with regard to the metabolic action of thyroid hormone in poikilotherms and its action in differentiating forms of such animals.

Material and Methods

Oxygen consumption was measured in a continuous-flow apparatus of the type used by Job (1955) in measuring the "standard" metabolism of trout. Four respirometer flasks (2.5-liter Fernbach culture jars) were used at any one time, one of these being used as a "blank." The four flasks were immersed in a large wooden tank in which the water level was maintained constant by means of an overflow. The flow of water through the flasks was so adjusted that *Squalus* embryos in groups of three removed about 0.5 to 1.0 ml. of oxygen. Rate of oxygen consumption was calculated from the flow rate, the difference in oxygen content between incurrent and excurrent water, and the wet weight of the animals

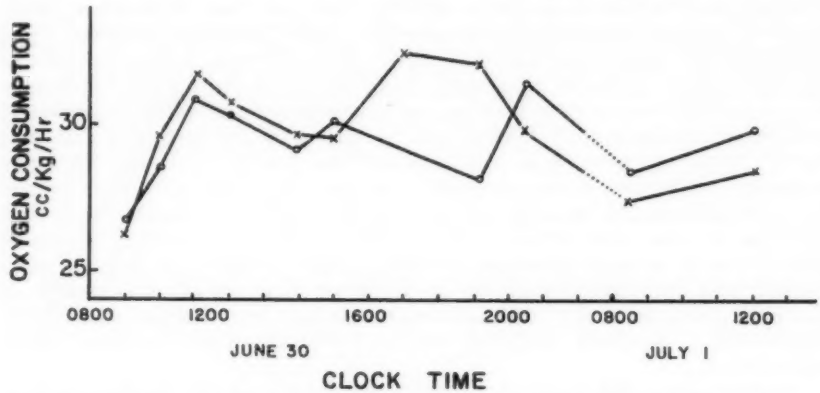

Figure 1. Serial determination of metabolic rate in untreated embryos of *Squalus suckleyi* over a 27-hour period. Two respirometers used with three pups in each.

tested. Oxygen content of the water was determined by the unmodified Winkler technique.

Water temperature never varied more than 1° C. during a single series of tests, and usually it did not change at all measurably. During most measurements of oxygen consumption the water temperature ranged from 13° to 14° C. However, extremes of 12° C. and 16° C. (on one unusually hot day) were recorded. The water in the large water bath was continuously aerated through a stone "air breaker," and preliminary tests showed that the oxygen content of the water was uniform at all points in the bath.

Embryos were tested in groups of three, being placed in the respirometers one hour before the first measurement of oxygen consumption. The bath containing the respirometers was covered to shield the animals from most of the light and other extraneous factors. Frequent observation indicated that under these conditions spontaneous muscular movements were rare. Several prelimi-

TABLE I

Metabolic rates of non-treated dogfish pups showing normal day-to-day variability in the laboratory. All runs made between 1:00 and 3:00 PM

Date	No. of trials	Mean O_2 consumption (cc./kg./hr.)
July 3	4	28.8 ± 1.4 (std. dev.)
July 4	4	31.4 ± 1.9
July 6	3	29.5 ± .89
July 8	3	31.3 ± 1.4
July 10	3	33.7 ± 1.7

nary measurements were made on untreated pups, to ascertain the variability in oxygen consumption under laboratory conditions. Figure 1 illustrates the results of serial determinations on two groups of embryos, all taken from the same female, over a 1½-day period. The period of steadiest metabolic rate was in the afternoon; accordingly all measurements reported here were made in the afternoon only. In another preliminary test, metabolic rates were determined on pups from one female dogfish, over a period of one week immediately following transfer of the pups to the laboratory. The results (Table I) show that the average metabolic rate remained at about the same level over this period and that the variability in oxygen uptake (as indicated by the standard deviations) was quite small.

TABLE II

Experimental protocol for each injection group. Numbers in parentheses indicate the number of pups from that female

Experiment	Total no. of injections	Females from which pups were taken	Substances tested*	Dose per injection
1	10	A(15)	triac	10 μg.
			l-Tx	10 μg.
2	9	B(11), C(9)	triac	10 μg.
			l-Tx	10 μg.
			PTU	50 μg.
3	9	D(10), E(10)	triac	10 μg.
				100 μg.
			l-Tx	10 μg.
			PTU	50 μg.
4	8	F(11), G(4), H(4), I(4), J(5), K(7)	triac	10 μg.
				100 μg.
			l-Tx	10 μg.
			T3	100 μg.
			PTU	50 μg.
5	5	L(19)	l-Tx	100 μg.
			T3	100 μg.
			PTU	50 μg.
6	4	M(25), N(10)	triac	10 μg.
			l-Tx	10 μg.

* Abbreviations: triac, triiodothyroacetic acid; l-Tx, l-thyroxine; PTU, propylthiouracil; T3, triiodothyronine.

TABLE III

Effects of repeated injections of thyroid hormones on oxygen consumption (cc./kg./hr.) of embryos of Squalus suckleyi.* *Values in the table are means of 3 to 6 consecutive determinations, taken at 15-minute intervals. E = experimental. C = control*

Experiment	No. of injections		Triac 10 μg.	Triac 100 μg.	l-Tx 10 μg.	l-Tx 100 μg.	T3 100 μg.	PTU 50 μg.
		E	32.40		33.52			
	1	C	30.79		32.52			
		E/C × 100	105		103			
		E	52.25		44.78			
1	4	C	39.28		39.28			
		E/C × 100	133		114			
		E			46.82			
	9	C			44.60			
		E/C × 100			105			
		E	32.25		32.15			28.78
	2	C	31.52		31.52			31.52
		E/C × 100	102		102			91
		E	41.90		40.90			36.00
2	4	C	35.80		35.80			35.80
		E/C × 100	117		114			101
		E	33.80		41.40			31.27
	9	C	34.16		34.16			34.16
		E/C × 100	99		121			91
		E	33.10	33.40	33.30			32.70
	1	C	33.20	33.20	33.20			33.20
		E/C × 100	99	101	100			98
		E	36.70	33.90	29.20			29.00
3	4	C	30.40	30.40	30.40			30.40
		E/C × 100	121	108	96			98
		E	35.87	35.50	33.62			38.09
	9	C	32.96	32.96	32.96			32.96
		E/C × 100	109	108	102			115
		E	34.13	32.56	30.82		30.12	30.30
	2	C	29.86	29.86	29.86		29.86	29.86
		E/C × 100	114	109	103		101	102
		E	37.02	33.45	33.97		34.63	32.51
4	5	C	30.41	30.41	30.41		30.41	30.41
		E/C × 100	122	110	112		114	107
		E	32.33	33.66	31.86		32.28	29.44
	8	C	27.49	27.49	27.49		27.49	27.49
		E/C × 100	118	122	116		118	107

* This table includes absolute values for oxygen consumption at the beginning (after 1 or 2 injections), the middle (after 4 or 5 injections), and end after 8 or 9 injections in each experiment. The complete course of each experiment is shown in Figures 4A, 4B and 4C, but these do not show absolute values. To tabulate all the absolute values would require an impractically long table.

TABLE III—*Continued*

Experiment	No. of injections		Triac 10 μg.	Triac 100 μg.	l-Tx 10 μg.	l-Tx 100 μg.	T3 100 μg.	PTU 50 μg.
5	1	E				30.01	25.78	26.96
		C				26.86	22.82	22.82
		E/C × 100				112	113	118
	5	E				39.92	42.96	37.07
		C				35.78	35.78	35.78
		E/C × 100				112	120	104
6	1	E	28.49		30.37			
		C	26.18		26.18			
		E/C × 100	109		110			
	4	E	35.34		32.82			
		C	29.78		29.78			
		E/C × 100	119		110			

The animals used were "pups" removed from the uteri of *Squalus suckleyi* females caught during July and August, 1958, at Friday Harbor, Washington, within 200 yards of the laboratory. The ovoviviparous young of this species remain in the uterus for two years. It could be estimated from the sizes of the yolk sacs that the embryos we used were approximately 19–23 months of age. Occasional "spontaneous" birth of pups of captive females was observed in late August. Embryos removed from the uterus were kept in apparently good condition for periods as long as several weeks in large, covered glass dishes (2-liter capacity) in groups of 4 or 5, in slowly flowing sea water. Whenever possible, all pups used in an experiment were taken from the same mother. In experiments requiring large numbers of pups it was necessary to combine litters from several mothers and these were distributed as evenly as possible into the different experimental groups (Table II). The limited number of embryos of equivalent development available at one time, and the limited capacity of the respirometers made it impossible to test all hormones at the same time. For this reason, six different experiments were performed during an eight-week period.

In each experiment groups of embryos were injected intraperitoneally on alternate days with various doses (Table II) of hormones, propylthiouracil, or 0.7% NaCl solution, always in a volume of 0.05 cc. Oxygen consumption was determined on the day after injection to avoid possible responses to handling.

The compounds tested for their effect on oxygen consumption were l-thyroxine (Tx), l-triiodothyronine (3:5:3′-triiodo-l-thyronine, T_3), triiodothyroacetic acid (3:5:3′ triiodothyroacetic acid, Triac) in doses of 10 micrograms or 100 micrograms, and propylthiouracil in doses of 50 micrograms.

EXPERIMENTS AND RESULTS

Six experiments (Table III) were completed. The total number of injections, given at 2-day intervals, was as few as 4 or 5, but was usually (in four of the six experiments) 8 to 10. The shorter experiments were ended when accidental

blockage of the sea water occurred. Since such occurrences were obviously harmful, and their effects difficult to assay, respiratory measurements were accordingly not continued. In experiment 1 such a blockage killed all Triac-injected animals after the fifth injection (Fig. 2). The Tx-injected animals in this experiment showed an extreme but temporary respiratory depression (Fig. 2) at the same time, so may have had a brief experience of the same nature. Only one other extremely variant datum is seen in Figure 3, which shows the results of experiment 2. Here an exceptionally high respiratory rate was observed in saline solution-injected controls after three injections. Since these same animals in succeeding measurements showed relatively little variation in oxygen consump-

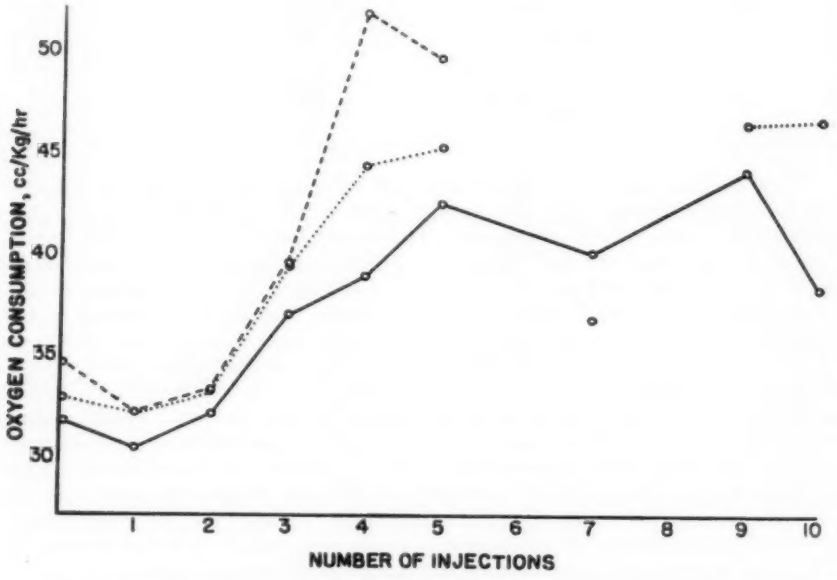

FIGURE 2. The effects of repeated injections of thyroid compounds on metabolic rate of dogfish embryos—injection group 1. Solid line, control; dashed line, triiodothyroacetic acid (10 μg.); dotted line, l-thyroxine (10 μg.).

tion, it is felt that the exceptional figure may have been due to some limited experience, possibly a brief interruption in water supply. The remaining data, summarized in the figures, and Table III, appear to show consistent trends of response, or lack of response, to the various forms of treatment.

It may be seen in Figures 1 and 2, and Table III, that the oxygen consumption of control embryos varied throughout the periods of respiratory measurement, but not in any particular pattern. The nature of this variation, whether due to maturational or environmental factors, is not clear. However, whatever the basis for the variation in control respiratory metabolism, the changes were generally gradual. In the first experiment (Fig. 2) oxygen consumption increased gradu-

ally through most of the three-week period of observation; in the second experiment (Fig. 3) this variation was, in general, less and showed no such constant trend.

Fortunately, the general variation of the controls was paralleled by the hormone-injected embryos, and in addition, a relative difference from the controls was usually maintained, if it occurred at all. Accordingly, when the results are expressed as per cent of the control oxygen consumption some conclusions appear to be offered (Table III, Fig. 4).

The most potent stimulator of oxygen consumption in these tests was triiodothyroacetic acid (Fig. 4A). Despite all the variations to which such experiments seem to be prone, in all four experiments in which Triac was injected in 10-microgram quantities, it clearly induced an increase in oxygen consumption to maxima 17% to 33% above the control. These maxima were achieved 8 to 10 days after beginning the injections and thereafter oxygen consumption progressively

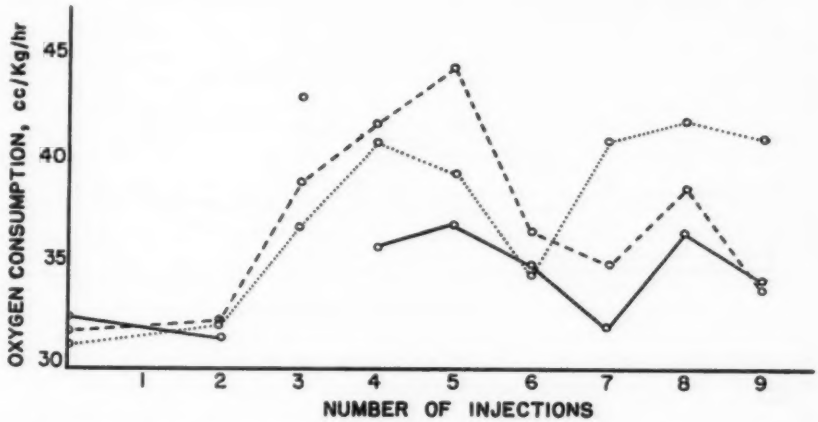

FIGURE 3. The effects of repeated injections of thyroid compounds on metabolic rate of dogfish embryos—injection group 2. Solid line, control; dashed line, triiodothyroacetic acid (10 μg.); dotted line, l-thyroxine (10 μg.).

decreased. The larger dose of Triac (100 micrograms) was less effective than the smaller one (Table III).

Thyroxine, in 10-microgram doses, was not as clearly a stimulator of oxygen consumption in the *Squalus* pups as was Triac. It consistently raised oxygen consumption in two of four experiments (Table III, Fig. 4B) above that of controls, but failed to do so in one, and in another did not produce a significant stimulation until the very end. However, in all thyroxine experiments oxygen consumption was rising at the end of the period of treatment, in comparison with the controls. In one test with 100-microgram quantities of thyroxine a 12% increase above the controls in respiratory metabolism was noted.

Triiodothyronine, in 100-microgram quantities, in all instances stimulated oxygen consumption to levels as high as 18% to 20% above the controls (Table III).

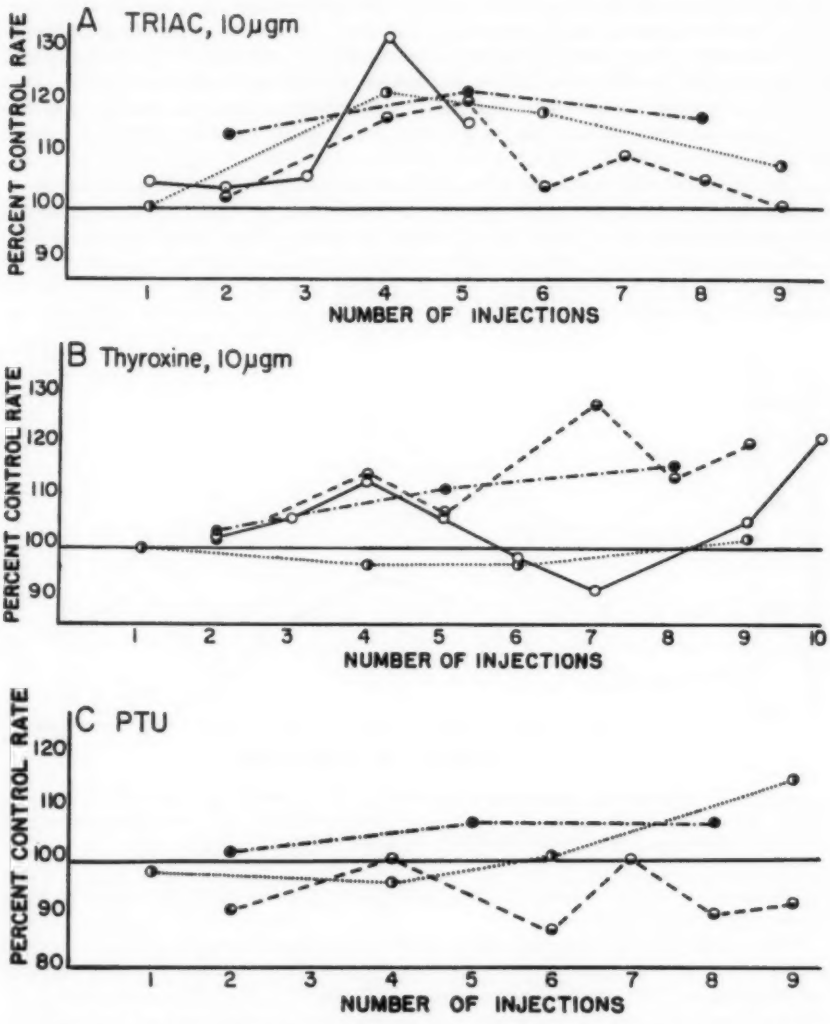

FIGURE 4; A, B, and C. The effects of repeated injections of thyroid compounds on metabolic rate of dogfish embryos. A, triiodothyroacetic acid, 10 µg. each injection; B, l-thyroxine, 10 µg.; C, propylthiouracil, 50 µg. Points represent per cent of the control rate. Open circles, injection group 1; horizontal-barred circles, injection group 2; vertical-barred circles, injection group 3; filled circles, injection group 4.

Propylthiouracil had no particular effect on oxygen consumption relative to the saline-injected controls (Table III, Fig. 4C).

Discussion

Results of this investigation indicate that, under the conditions of these experiments, 10 micrograms of triiodothyroacetic acid (Triac), given on alternate days, have acted as a stimulant of oxygen consumption in the near-term shark embryo (Fig. 4A). The unusual feature of this response is its diminution after 8 to 10 days despite continued injections of the hormone. In laboratory mammals (Foster, Palmer and Leland, 1936) and in clinical use (Means, Lerman and Salter, 1933) the continued administration of thyroxine or crude thyroid preparations is usually accompanied by a sustained increased respiratory rate. However, even in clinical experience it has been reported (Eppinger and Salter, 1935) that an initial rise in metabolism following a week of treatment with thyroid hormone may be followed by a sharp drop, even though treatment is continued. Thus, this pattern of metabolic response is not unprecedented, and may depend on particular physiological factors involved in the response to thyroid hormone. It is of especial interest that Triac has been found to be about 10 to 25 times as active as thyroxine in stimulating amphibian metamorphosis (Pitt-Rivers and Tata, 1959). A ten-fold larger dose of Triac was no more active than the 10-microgram quantity, and appeared, in fact, less active (Table III).

Thyroxine, in either the 10- or 100-microgram dosage, was less clearly a metabolic stimulant. In two of four experiments animals receiving the 10-microgram dose remained consistently higher than controls in oxygen consumption by some 10% to 20%. In the other two experiments this superiority was either lacking or irregularly variable. However, in all four instances oxygen consumption was rising (relative to the saline-injected controls) at the ends of the experiments (Fig. 4B). This was the most variably effective metabolic stimulant and no explanation can be offered for this variability. Larger doses of thyroxine and triiodothyronine (Table III) produce a 12 to 20% increase in oxygen consumption by 10 to 16 days after beginning the injections of hormone.

Propylthiouracil was neither a stimulant nor depressant for oxygen consumption in four different experiments which lasted about 18 days each. Almost all measures of oxygen consumption in dogfish pups treated with this antithyroid drug were within 10% of the control. It has been reported by Zaks and Zamkova (1952) that thiourea consistently reduces the oxygen consumption of young salmon and sturgeons below that of controls. Chavin and Rossmore (1956), working with thiouracil-treated young goldfish, found no effect on oxygen consumption. Interpretation of results of treatment with antithyroid drugs is always complicated by the fact that they are known to be toxic, even in small doses. Since the absence of a metabolic response to propylthiouracil might mean merely that no hormone is yet produced by the thyroid of these embryonic animals, five of them were injected with radioiodide (5 microcuries) and the rest were fixed for histological examination to investigate this possibility. The 24-hour thyroidal radioiodine uptake varied from 0.25% to 2%, a small but significant degree of accumulation. This compares favorably with thyroid uptakes of about 1.5% found by Gorbman and Waterman (unpublished) in pups of the Atlantic spiny

dogfish, *Squalus acanthias*. Vivien and Rechenmann (1954) who also treated shark pups (*Scyliorhinus canicula*) with I^{131}, observed by radioautographic techniques that it is deposited in the thyroid, presumably in protein-bound form. The thyroid tissue examined histologically showed a slight increase in average cell height (about 25%) and "vacuolization" of the colloid. This would appear to indicate that the pituitary-thyroid axis of mutual responsiveness is differentiated in these animals, and that it had responded in the PTU-treated animals to a change in thyroid hormone output by TSH secretion. However, despite this apparent decrease in endogenous thyroid hormone production there was no detectable change in oxygen consumption. It is possible that this decrease in endogenous thyroid hormone, if real, was much smaller in size than the 10 micrograms in the injected dose.

In summary, it may be said that triiodothyroacetic acid has been shown in these experiments to be a temporary stimulant of oxygen consumption in near-term embryos of *Squalus suckleyi*. Triiodothyronine proved slightly less active, and the metabolic stimulation by thyroxine was irregular. The thyroid glands of these animals appeared to be functioning at a low rate, and interruption of this function by propylthiouracil had no demonstrable effect on oxygen consumption.

Summary

1. The oxygen consumption rate of "near-term" pups of the dogfish, *Squalus suckleyi*, was determined at regular intervals during the course of repeated injections of physiological saline solution, thyroid hormones, or of anti-thyroid substances. Up to 10 injections were given on alternate days.

2. Of the compounds tested, triiodothyroacetic acid at a dosage level of 10 micrograms per injection was the most consistent in raising the level of oxygen consumption. The effect, however, was transitory with oxygen consumption rising to a maximum (17% to 33%) level above the saline-injected controls after four injections, thereafter declining slowly to control levels.

3. L-thyroxine at a dosage level of 10 micrograms had a variable effect on oxygen consumption. In two of four experiments the oxygen consumption rate rose irregularly, reaching a level about 20% above the controls after 9–10 injections. In the remaining experiments, there was no clear tendency to remain above the controls.

4. Propylthiouracil, after 9 injections, had no consistent effect on metabolic rate in four experiments.

5. The results are discussed with reference to the possible level of thyroid function in these animals.

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PERMEATION AND MEMBRANE TRANSPORT IN PARASITISM: STUDIES ON A TAPEWORM-ELASMOBRANCH SYMBIOSIS [1]

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It was shown by Read, Simmons and Rothman (1960) that the amino acids L-valine and L-leucine enter the tapeworm, *Calliobothrium verticillatum*, by a process showing adsorption kinetics. The data obtained ruled out simple diffusion but did not permit a definite conclusion as to whether the permeation is a process of active transport. L-valine and L-leucine were each shown to competitively inhibit the entry of the other into the worm. Several other amino acids were shown to inhibit L-valine and L-leucine permeation, but it was not established that these inhibitions were competitive. The studies to be reported have shown that L-valine is actively transported, although further investigation has revealed that when *Calliobothrium* is treated as a member of a symbiotic relationship, the operation of the amino acid entry systems is indeed complex. Appreciation is expressed to Dr. G. Wertheim who lent technical assistance during part of this study, and to the gentlemen of the Supply Department of the Marine Biological Laboratory, Woods Hole, Massachausetts, who furnished many living dogfish.

Materials and Methods

The methods of collection and handling of *Calliobothrium* from the dogfish, *Mustelus canis*, were similar to those used in a previous study (Read, Simmons and Rothman, 1960). The salt solution used in handling both worms and host tissues had the following composition: NaCl, 250 mM.; KCl, 4.4 mM.; $CaCl_2$, 5.1 mM.; $MgCl_2$, 2.9 mM.; urea 300 mM.; and tris (hydroxymethyl) amino methane-maleate buffer, 10 mM. (pH 7.2). All incubations were carried out in this medium, with appropriate experimental additions, at 10° C. In preparing tissues for experimental incubation, the worms were washed in several changes of the salt solution and incubated for 60 minutes at 10° C. before an experiment. Host tissues were removed in ice-cold salt solution and, before the experiment, were incubated at 10° C. for 30 minutes in a large volume of salt solution containing 10 mM. glucose. Throughout this period, the tissue was vigorously aerated with a sintered bubbler attached to a small air pump. For experimental incuba-

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tion, the worms or host tissues were lightly blotted on hard filter paper and transferred to the experimental medium. At the end of an experimental incubation, the worms or host tissues were rinsed by dipping twice in large volumes of the salt solution, blotted quickly on hard filter paper, and placed in a measured volume of 70% ethyl alcohol. It was previously shown that, with occasional shaking, free amino acids are extracted from worm tissues in 50 to 70 per cent alcohol in less than 24 hours (Read, Simmons and Rothman, 1960). In most cases extraction was carried out for over 48 hours but in no case for less than 24 hours. Actually, the concentration in the fluid of the tissues probably comes to equilibrium with the alcohol external to the tissues. The quantity of worm tissue with respect to the volume of the extracting fluid was kept sufficiently low so that an error of less than 1% was introduced by the addition of the worm volume to that of the extracting fluid. Aliquots of alcoholic extracts were used for determination of radioactivity or analysis of amino acids.

Many of the data are expressed in terms of the alcohol-extracted dry weight of tissue; this was determined by heating the extracted tissue for 5 to 6 hours at 100° C. in tared foil pans. Drying for longer periods produced no significant change in dry weight values. Determinations of the alcohol-extracted dry weight/wet weight for 16 worms at the end of a 60-minute incubation in the salt solution at 10° C. gave a value of 0.302 ± 0.016. Nineteen dogfish gut samples, identical with those used in experiments, gave an alcohol-extracted dry weight/wet weight value of 0.135 ± 0.011 after a 30-minute, aerated incubation in salt solution with glucose at 10° C. Plating of extract samples and determinations of radioactivity were carried out as previously described (Read, Simmons and Rothman, 1960).

Two-dimensional chromatography was carried out by a modification of the method of Levy and Chung (1953), as described by Campbell (1960). One-dimensional chromatograms were prepared on Whatman No. 52 paper using sec-butyl alcohol, formic acid, and water (75:15:10) as the solvent system. Amino acids were quantitatively estimated using the methods of Fowden (1951). Radioautographs of chromatograms were prepared by exposing Eastman "no-screen" x-ray film to the chromatograms after removing the solvent. Histidine was determined by the method of Macpherson (1946). Nitrogen was determined by the micro-Kjeldahl method described by Lang (1958). Other details of methods will be described in context.

Experimental

Further characterization of the amino acid entry systems of Calliobothrium

Several amino acids have been shown to inhibit the penetration of L-valine and L-leucine into *Calliobothrium* (Read, Simmons and Rothman, 1960). In the present study a number of experiments were carried out to determine whether these inhibitions are competitive in nature, and whether there is a reciprocal inhibitory effect of L-valine on penetration of certain of the inhibitory amino acids. An analysis of the inhibitory effects of L-serine, L-threonine, and L-alanine on valine entry showed that the inhibitions indeed are competitive in nature (Table I). Conversely, experimental analysis of the effect of L-valine on the

TABLE I

Effect of L-serine, L-threonine, and L-alanine on the entry of L-valine into Calliobothrium. *S = concentration of L-valine; V = counts per minute per gram of alcohol-extracted dry tissue; N = number of samples*

Amino acid	Inhibitor	N	1/S	V
L-valine-C14	$5 \times 10^{-3}M$ L-serine	4	200	59,575 ± 3673
		4	500	36,200 ± 2876
		4	1000	18,212 ± 1004
		4	2000	11,515 ± 1529
	$2 \times 10^{-3}M$ L-serine	4	200	96,900 ± 4300
		4	500	41,850 ± 1947
		4	1000	24,300 ± 571
		4	2000	16,812 ± 857
	None	4	200	116,600 ± 10,148
		4	500	61,222 ± 5793
		4	1000	46,700 ± 2200
		4	2000	31,775 ± 1520
L-valine-C14	$5 \times 10^{-3}M$ L-threonine	4	200	65,666 ± 4843
		4	500	36,368 ± 6319
		4	1000	18,769 ± 852
		4	2000	10,042 ± 1102
	$2 \times 10^{-3}M$ L-threonine	4	200	73,450 ± 5576
		4	500	45,300 ± 4529
		4	1000	27,937 ± 1971
		4	2000	16,875 ± 1639
	None	4	200	93,725 ± 7925
		4	500	64,550 ± 5794
		4	1000	40,920 ± 3415
		4	2000	28,300 ± 3706
L-valine-C14	$5 \times 10^{-3}M$ L-alanine	4	200	53,825 ± 3594
		4	500	31,070 ± 2400
		4	1000	22,400 ± 842
		4	2000	10,100 ± 597
	None	4	200	91,550 ± 4132
		4	500	75,902 ± 2926
		4	1000	41,850 ± 4903
		4	2000	28,475 ± 2685

penetration of L-serine demonstrated that valine competitively inhibits the entry of this amino acid (Table II).

Previous studies showed that at a concentration ratio of 2:1, glutamic acid did not inhibit the entry of L-valine. Therefore, it was surprising to find that aspartic acid is an effective inhibitor of L-valine entry. With L-valine at a concentration of 2×10^{-3} *M*, aspartic acid or proline at a concentration of 5×10^{-3} *M*, inhibited L-valine entry an average of 33 and 45%, respectively, in five experiments. A shortage of experimental material prevented a determination of whether or not aspartic acid is a competitive inhibitor.

L-lysine-C14 penetrates *Calliobothrium* at a very low rate. A series of experiments were performed to determine whether L-lysine or L-valine affect the entry of the other. L-lysine entry was not significantly affected by L-valine and L-valine entry was not affected by L-lysine (Table II). The apparent stimulation of L-valine entry by L-lysine at a lysine/valine ratio of 2, previously reported (Read, Simmons and Rothman, 1960), is not considered to be significant in view of the results obtained when this broader range of concentration ratios was examined.

When *Calliobothrium* was incubated for two minutes in 5×10^{-3} *M* L-valine-C14 and subsequently incubated for additional periods in salt medium without

TABLE II

Effects of certain amino acids on entry of L-serine, L-valine, and L-lysine. Data presented as in Table I

Amino acid	Inhibitor	N	1/S	V
L-serine-C14	$5 \times 10^{-3}M$ L-valine	4	200	45,600 ± 6111
		4	500	29,500 ± 8201
		4	1000	19,100 ± 1124
		4	2000	11,460 ± 823
	None	4	200	57,433 ± 2441
		4	500	42,150 ± 3300
		4	1000	29,100 ± 629
		4	2000	20,200 ± 4540
L-valine-C14	$5 \times 10^{-3}M$ L-lysine	4	200	105,975 ± 13,192
		4	500	73,050 ± 2563
		4	1000	47,300 ± 3379
		4	2000	32,750 ± 2958
	$2 \times 10^{-3}M$ L-lysine	4	200	97,025 ± 12,604
		4	500	72,025 ± 3620
		4	1000	46,250 ± 2418
		4	2000	31,433 ± 2240
	None	4	200	103,775 ± 14,202
		4	500	61,300 ± 963
		4	1000	47,300 ± 2588
		4	2000	34,050 ± 2952
L-lysine-C14	$5 \times 10^{-3}M$ L-valine	4	200	14,970 ± 1207
		4	500	7552 ± 1965
		4	1000	6247 ± 1406
		4	2000	5410 ± 441
	$2 \times 10^{-3}M$ L-valine	4	200	15,115 ± 3924
		4	500	7997 ± 1534
		4	1000	6713 ± 1408
		4	2000	5290 ± 805
	None	4	200	15,358 ± 1665
		4	500	7930 ± 1262
		4	1000	6232 ± 1836
		4	2000	5142 ± 909

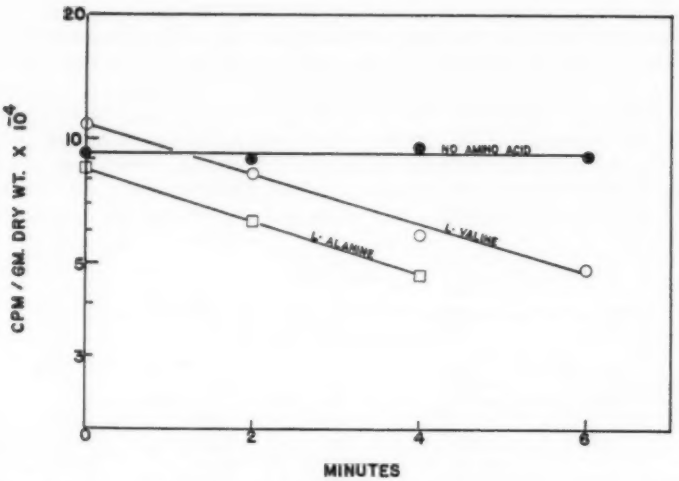

FIGURE 1. Loss of L-valine-C14 from *Calliobothrium* in the presence and absence of unlabeled amino acid in the suspending medium.

amino acid, "leakage" of L-valine from the tissues did not occur. However, when the worm, containing labeled amino acid, was placed in salt medium containing 5×10^{-3} *M* unlabeled L-valine, a decrease in the C14-labeled valine content of the worm was observed. This was analyzed by varying the time of incubation of labeled worms in medium containing unlabeled amino acid. The loss of labeled amino acid from the tissues followed a decay curve. A similar loss of L-valine-C14 from the worm occurred when the external medium contained unlabeled L-alanine (Fig. 1). In 6 experiments, incubation of worms in 5×10^{-3} *M* L-valine for 60 minutes had no effect on the entry rate of labeled valine in a subsequent two-minute period.

During the present study attempts were made to determine whether L-valine is concentrated against an electrochemical gradient. Worms were incubated for 40 minutes in L-valine-C14. The medium and the tissues were analyzed for free valine. The concentrations of valine inside and outside the worms are shown in

TABLE III

The accumulation of L-valine by Calliobothrium *against a concentration gradient. Incubation in* 9×10^{-3}*M L-valine for 40 minutes at 10° C.*

Sample	Final concentration in medium μM valine/ml.	Final concentration in worms μM valine/g. water	Worm/Medium
1	6.5	16.9	2.6
2	5.5	23.7	4.3
3	6.3	19.2	3.0
4	7.2	21.3	2.9

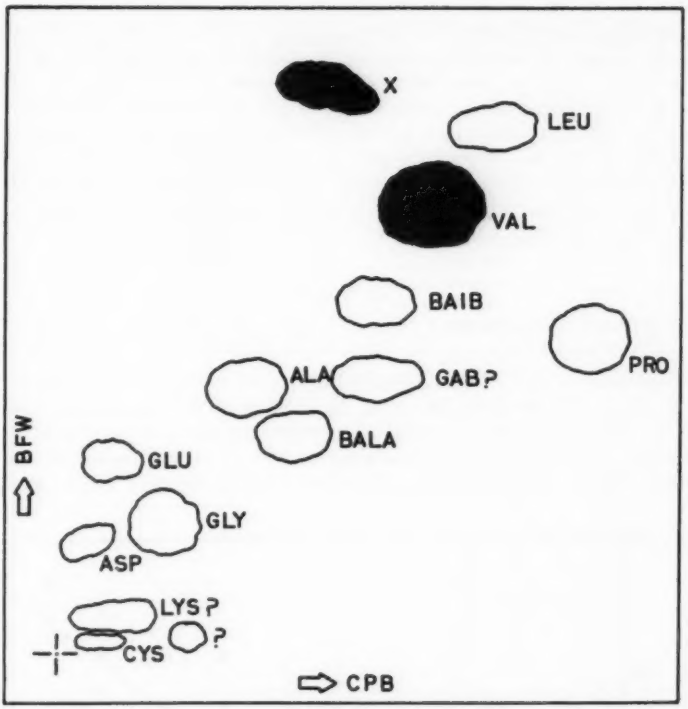

FIGURE 2. Chromatogram of free amino acids extracted from *Calliobothrium* after 40 minutes' incubation in presence of L-valine-C14. Solvent systems were sec-butyl alcohol, formic acid, water (75 : 15 : 10) = BFW and meta-cresol, phenol, borate buffer, ph 8.3 (60 : 30 : 15) = CPB. Black areas show presence of radioactivity, X being a non-ninhydrin-positive, unidentified metabolite.

Table III. It is apparent that valine is concentrated against a gradient. Radioautographs prepared from the chromatograms of tissue extract from the 40-minute incubations revealed that some L-valine is metabolized in this period. However, L-valine was the only radioactive ninhydrin-positive compound on the chromatograms. A second radioactive spot was present but did not react with ninhydrin. A representative chromatogram is shown in Figure 2. It was not feasible to characterize the unknown metabolite further. On the other hand, radioautographs prepared from 2-dimensional chromatograms of worm extracts from 2-minute incubations in L-valine-C14 revealed a single radioactive spot which proved to be identical with valine by the "fingerprint" method.

The amino acid entry systems of the host gut

Agar *et al.* (1956) differentiated and studied (1) the absorption of amino acids by the rat intestine *in vivo;* (2) the transfer of amino acids from inner to

TABLE IV

Uptake of L-valine-C14 by spiral valve tissue of Mustelus *spiral valve. Incubation in* 5×10^{-3} *M L-valine for 2 minutes at 10° C. Values are counts per minute per gram alcohol-extracted dry weight*

Spiral No. 2	Spiral No. 6
80,300	79,300
85,900	89,600
93,500	65,100
74,700	86,500
79,000	88,900
87,000	80,600
88,800	90,000
82,000	
Mean 83,900	82,857

outer fluids using loops of rat intestine; and (3) the uptake of amino acids by intestinal tissue. In short experiments, the kinetics of the latter two showed rather good agreement, although, as might be expected, a lag was observed in transfer experiments. Since the removal of amino acids from the lumen of the gut by tapeworms and by the host mucosa, rather than transport of amino acids in the

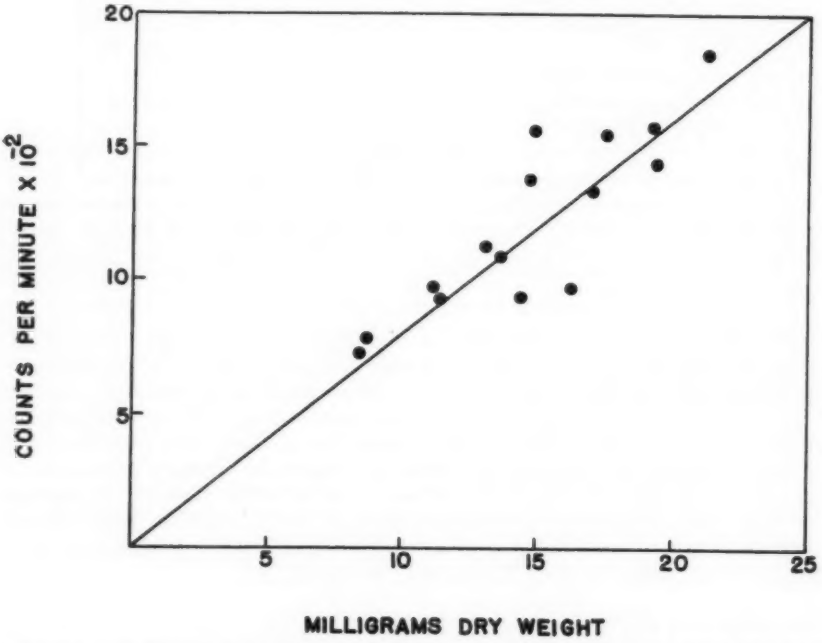

FIGURE 3. The quantity of labeled valine taken up by various amounts of spiral valve tissue of *Mustelus*. Tissue weight is alcohol-extracted dry weight.

extra-intestinal host tissues, is the primary biological aspect to be considered here, experiments to study the uptake of amino acids by dogfish intestinal tissues were carried out. The preparations used were pieces from the lamina of the spiral valve cut approximately 1 cm. square. As many as 30 such pieces can be obtained from the lamina of a single spiral turn in the intestine of a sexually mature dogfish. Most of the experiments to be described were carried out with tissues from the fourth spiral posterior to the pyloric valve, although, as will be shown below, preparations from other spirals would probably yield quite comparable data. *Calliobothrium* is found predominantly in the region of the fourth and fifth spirals. The methods of handling the dogfish tissue were described earlier. It was reasoned that if preparations from different parts of single or separate spiral lamina of the intestine showed consistency in rate of amino acid uptake, with respect to weight of tissue, replicate samples from a single fish could be used in amino acid uptake studies. Initially, therefore, a determination of amino acid uptake was made with tissue samples removed from the second and sixth spirals. Data obtained with tissues from a single fish are presented in Table IV and show remarkably that there is no significant difference in the entry of L-valine into tissues from these two regions of the intestine nor into tissues from different parts of the same lamina. Further, the amount taken up is proportional to the dry weight of tissue used (Fig. 3). These findings showed that multiple sampling for kinetic studies is feasible with these preparations.

TABLE V

Reciprocal inhibition of L-serine and L-valine entry and L-lysine entry into spiral valve tissue of Mustelus canis. *Data presented as in Table I. For experimental details, see text*

Amino acid	Inhibitor	No.	1/S	V
L-serine-C14	None	4	200	42,000 ± 3725
		4	500	28,700 ± 4340
		4	1000	14,900 ± 1859
		4	2000	7695 ± 1236
	$5 \times 10^{-3}M$ L-valine	4	500	10,666 ± 1231
		4	1000	6056 ± 586
		4	2000	2966 ± 561
L-valine-C14	None	4	200	68,980 ± 8271
		4	500	35,630 ± 6643
		4	1000	21,996 ± 5036
		4	2000	13,286 ± 1306
	$5 \times 10^{-3}M$ L-serine	4	200	32,666 ± 2714
		4	1000	15,860 ± 2286
		4	2000	9760 ± 1699
L-lysine-C14	None	4	200	27,293 ± 646
		4	500	17,551 ± 3409
		4	1000	10,800 ± 1184
		4	2000	6575 ± 1197

TABLE VI

Effects of other amino acids on entry of L-valine into spiral valve tissue. V = counts/min./gram alcohol-extracted dry tissue. N = Number of experiments

Inhibitor	N	V
Glycine	4	98,771 ± 6129
L-leucine	4	57,692 ± 3406
L-isoleucine	4	44,326 ± 1267
L-methionine	4	40,253 ± 3294
L-threonine	4	71,655 ± 4029
L-lysine	4	79,363 ± 2326
None	4	91,799 ± 1079

When the concentration of L-valine was varied, it was found that the entry of the amino acid into mucosal tissues follows an adsorption isotherm, and the apparent Michaelis constant for valine entry is about 5×10^{-3}. The addition of unlabeled L-serine at a concentration of 5×10^{-3} produced an inhibition of L-valine entry which was competitive in nature (Table V.) Conversely, L-valine competitively inhibits L-serine entry (Table V) which has an apparent Michaelis constant of about 4.5×10^{-3}.

A number of other amino acids were tested as inhibitors of L-valine entry. Data obtained are summarized in Table VI. It was found that "preloading" the mucosa, by incubating for 40 minutes in non-radioactive L-valine, produced no effect on the subsequent entry rate of L-valine-C14. In balanced salt solution without added non-radioactive amino acid, L-valine does not leak out of the intestinal tissue to a significant extent.

TABLE VII

Relative concentrations of free amino acids in the fluid contents of the spiral valve of Mustelus canis. *All values are related to a valine concentration of 1.00. mg. N = Mg. of alcohol-soluble nitrogen in the sample*

Amino acid	Dogfish No.												$\bar{X} \pm$ S.E.
	1	2	3	4	5	6	7	8	9	10	11	12	
Leucine	1.43	1.46	1.20	1.53	1.43	1.67	1.44	1.15	1.18	1.38	1.61	1.41	1.41 ± 0.047
Phenylalanine	0.77	0.77	0.53	0.71	0.47	0.58	0.62	0.54	0.84	0.57	0.75	0.59	0.64 ± 0.034
Valine	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Tyrosine	0.76	0.91	0.55	0.94	0.43	0.65	0.82	0.63	0.90	0.50	0.75	0.70	0.71 ± 0.049
Alanine	1.35	0.78	0.87	1.94	1.50	1.42	1.65	1.21	1.13	1.25	1.66	1.51	1.36 ± 0.096
Threonine	0.58	0.42	0.35	0.65	0.78	0.49	0.88	0.75	0.83	0.72	1.07	0.84	0.70 ± 0.060
Glutamic	1.98	1.34	1.61	4.24	2.66	3.63	7.65	2.70	2.42	1.99	2.87	2.92	3.00 ± 0.49
Glycine & serine	1.61	1.18	1.72	5.41	2.90	3.12	4.61	2.41	6.26	4.13	3.44	3.14	3.33 ± 0.45
Aspartic	0.95	0.67	1.11	2.47	1.52	1.43	3.06	1.15	2.60	3.50	1.38	3.46	1.94 ± 0.29
Lysine	1.37	1.00	1.70	5.77	7.05	2.27	1.85	1.78	2.04	3.32	6.97	2.44	3.13 ± 0.63
Histidine	0.51	0.48	0.73	—	—	0.77	1.01	0.69	1.09	0.33	1.61	0.51	0.64 ± 0.095
Arginine	0.46	0.57	0.53	—	—	0.39	0.82	0.60	0.86	0.35	0.59	0.33	0.46 ± 0.078
Cysteine	—	—	—	—	0.84	0.70	—	0.52	—	—	—	0.21	0.19 ± 0.090
Beta aminoisobutyric	—	—	—	—	0.18	0.19	—	0.34	—	0.26	—	—	0.081 ± 0.037
Beta alanine	—	—	—	—	—	0.24	1.94	2.21	0.99	0.55	1.44	—	0.62 ± 0.23
mg. N	4.70	1.43	2.27	4.20	7.40	3.35	4.84	3.28	2.60	5.80	3.00	9.40	

Using dogfish gut preparations, attempts were made to show the inhibition of L-histidine uptake by other amino acids in experiments of 30-minute duration. L-histidine was taken up by the tissues and, during the experimental period, molar ratios of histidine in the tissue water/histidine in the external fluid of more than 2 developed. Initial concentration of histidine in external fluid was 1 mM. Addition of L-alanine, L-proline, L-valine, L-serine, or L-aspartic acid at a concentration of 2.5 mM. did not affect L-histidine uptake significantly. Histidine was removed from the medium at the rate of 20 to 40 micromoles per gram dry weight per 30 minutes.

Free amino acids of the intestinal lumen

Samples of the fluid contents of the spiral intestine were collected from a number of dogfish. There was great variation in the nutritional state of these animals. Some were freshly captured and some had been held in captivity for as long as 8 days without food. The samples were taken with a calibrated pipette from the middle portion of the spiral valve of living fish. The measured volume was immediately added to 10 volumes of 70% ethyl alcohol, mixed, and allowed to settle for several days. Free amino acids in the supernatant liquid were quantitatively determined. The analyses are summarized in Table VII. It is evident that there is great variation in the absolute quantity of alcohol-soluble nitrogen and of single amino acid components. However, study of the data shows that, for the most part, the molar ratios of one amino acid to another are strikingly constant. The dicarboxylic acids, aspartic and glutamic acid, show considerable variation, but it may be seen that the ratio aspartic/glutamic is relatively stable. The lysine/valine ratio also showed considerable variation. This may be associated with the relatively low rate at which lysine penetrates the mucosa. The methods used did not allow a clear separation of glycine and serine and these two amino acids were estimated together. However, it was roughly estimated that serine made up about 75 per cent of this value.

Discussion

The demonstration that certain amino acids, which inhibit the entry of L-valine into *Calliobothrium*, do so competitively, as previously shown with L-leucine (Read, Simmons and Rothman, 1960) suggests that this may also be the case with other inhibitory amino acids. Limitations of time and material have not allowed a complete analysis of the inhibitions of valine entry produced by cysteine, methionine, glycine, or proline.

Furthermore, some of the amino acids which do not inhibit the entry of L-valine or L-leucine at a concentration ratio of 2:1 may very well inhibit at higher concentration ratios. D-valine was found to inhibit L-valine entry at a very high D/L ratio but had no significant effect when D-valine/L-valine was 2 (Read, Simmons and Rothman, 1960). Of great interest is the failure of mutual competition between L-lysine and L-valine over a wide range of concentration ratios. Since L-lysine entry appears to follow adsorption kinetics, the lack of interaction with L-valine entry suggests that the two compounds enter at different sites.

The inhibition by other amino acids of L-valine and L-serine entry into the

intestinal tissue of the dogfish, shown to be mutually competitive in the case of L-serine and L-valine, is consistent with the observations of others who have reported inhibition of intestinal absorption of single amino acids by other amino acid species in warm-blooded vertebrates (Wiseman, 1955, 1956; Agar *et al.*, 1956).

A difference, however, is observed in the case of L-histidine uptake by dogfish and rat intestinal tissues. Agar *et al.* (1956) found that L-histidine uptake was markedly inhibited by addition of equimolar concentrations of a number of single amino acids. Inhibition was not observed with dogfish tissues when several amino acids were singly added at twice the histidine concentration. Christensen and his co-workers (reviewed by Christensen, 1959) have described reciprocal inhibitions of amino acid uptake with Ehrlich ascites tumor cells and such relationships between amino acids are known to occur with *Neurospora* (Mathieson and Catchside, 1955)

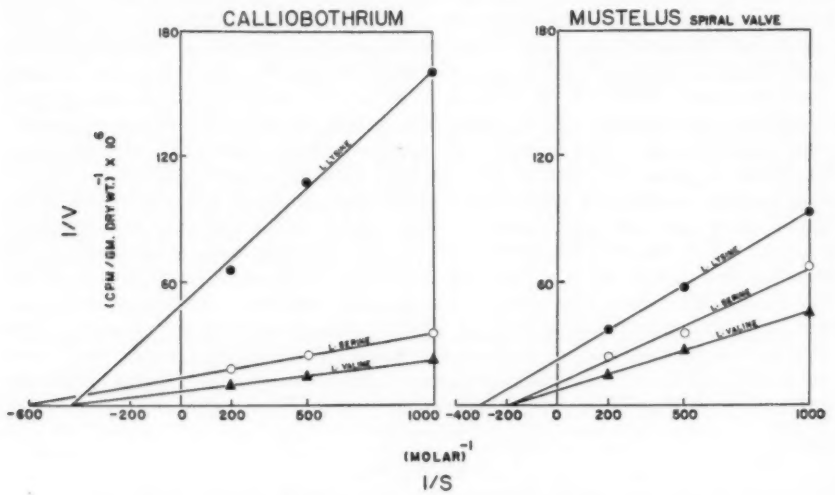

FIGURE 4. A kinetic comparison of the uptake of three amino acids by *Calliobothrium* and *Mustelus* spiral valve tissue.

and certain bacteria (Cohen and Monod, 1957). It appears to be a very general phenomenon, perhaps as general as the distribution of special membrane mechanisms for amino acid entry. It seems unfortunate that some of the bacterial physiologists have chosen to refer to such entry systems as "permeases," a term implying that there is an entry enzyme. It is to be hoped that a term with such specific connotations is not widely adopted until considerably more understanding of mechanism is attained.

There are definite differences in the amino acid entry systems of *Calliobothrium* and the dogfish intestinal tissue. In Figure 4 the worm and host tissue are compared with regard to their affinities for three amino acids.

Several years ago the senior author reviewed the literature on the physiology of the small intestine with special reference to its peculiarities as a habitat (Read,

1950). It was concluded that there is a flow of organic compounds, with the notable exception of carbohydrates, from the tissues into the gut lumen and that much of this material is resorbed in areas of the gut distal to the point of secretion. It seemed obvious to infer that many of these materials are available to lumen-dwelling parasites. The present data on the free amino acids in the gut lumen of dogfish in highly variable states of nutrition, and the great stability of molar ratios of these amino acids lend weight to the above concept. As a part of a study of mammalian nutrition, Nasset and his colleagues (1955) demonstrated that molar ratios of free amino acids, one to another, in the small intestine of the dog are astonishingly constant and independent of the composition of protein ingested. The concentration ratios were essentially unchanged in dogs receiving *no* protein by mouth. This has broad implications in considering intestinal parasitism. It reinforces the senior author's argument that in many chemical characteristics, the small intestine represents a relatively stable environment in a particular host. More specifically, it invites inquiry as to what effects constancy of the relative amounts of different amino acids might have on a particular intestinal parasite. It seems plain that if the environment offers a mixture of amino acids or other compounds of nutritional significance, and if these compounds compete with or otherwise affect the entry of one another into the tissues of the parasite, the ratios of amino acids in the mixture will be extremely important in determining whether the nutritional requirements of the parasite can be maintained in a balanced state. The concept emerges that the ratios of nutrients may be *critical* in determining whether or not a given mixture of amino acids will represent a satisfactory food for a parasitic organism such as a tapeworm. Thus, ratios of nutrient concentration may be critical limiting parameters at the *interface* between host and parasite. Further, the ratios of rates of entry of amino acids may be manifestations of an important regulatory system governing the make-up of the amino acid pool in a given worm.

As a homeostatic mechanism of importance in the physiology of the vertebrate, the competitions between amino acids would seem to represent part of a mechanism for regulating the composition of the amino acid mixture entering the portal system and hence the liver. Nasset (1957) has presented evidence that the relative concentrations of amino acids in the small gut are maintained by the secretion of endogenous nitrogenous material which is mixed with the ingesta. It would seem that the regulation of the composition of the amino acid pool for protein synthesis in the vertebrate begins at the mucosa.

While it would appear that the host and the parasite are competing with each other as whole organisms and, from this standpoint, the competition of worm and host should be considered in terms of total absorption by worm and intestine, we may consider highly localized competition in terms of the entry systems for a particular amino acid. If other amino acids affect entry of this amino acid into the mucosa and the worm to differing extents, it is apparent that the concentration ratios of amino acids may undergo alteration in the immediate vicinity of the worm-mucosa system. If the rates involved do not undergo marked change, a new set of concentration ratios should be established. If this is indeed true, it may have wider implications in considering parasitisms in which the most obvious effects on the host are general unthriftiness or ill defined interferences with nutrition. It has not been feasible to study this experimentally in the dogfish-cestode system

but it may be practical with other host-parasite combinations more amenable to laboratory control.

It becomes increasingly apparent that the gut should not be considered a space outside the vertebrate body. The rapid changes in the properties of intestinal mucus indicating hydrolysis of components (Hartiala and Grossman, 1952) suggest that secretion, hydrolysis, and resorption must occur constantly. Lumen parasites are in a position to remove from this exocrine-enteric circulation compounds of nutritional value. If the data on rate of entry of individual amino acids into *Calliobothrium* and host intestinal tissue are calculated on the basis of water content, making the assumption that the amino acids are in solution in this water, the tapeworm takes up the amino acids studied at a much higher rate than host intestinal tissue. However, the data on competitions indicate that rates of absorption for single amino acids are not directly applicable to complex mixtures. Study of entry of single components from complex mixtures is obviously required.

Summary

1. The entry of C14-L-valine into the tapeworm, *Calliobothrium verticillatum*, is competitively inhibited by L-serine, L-threonine, and L-alanine. Conversely, L-valine competitively inhibits the entry of C14-L-serine.

2. The entry of C14-L-valine is not significantly affected by L-lysine and, conversely, L-lysine entry is not affected by L-valine.

3. L-valine is concentrated against a gradient by *Calliobothrium* in experiments of 40-minute duration.

4. The entry of C14-L-valine into mucosal tissues of the dogfish host, *Mustelus canis*, is competitively inhibited by L-serine and, conversely, C14-L-serine entry is competitively inhibited by L-valine. L-leucine, L-isoleucine, L-methionine, L-threonine, and L-lysine also inhibit C14-L-valine entry but it has not been shown that inhibition is competitive.

5. In experiments of 30-minute duration, L-histidine uptake by dogfish mucosa was not affected by L-alanine, L-proline, L-valine, L-serine, or L-aspartic acid at the concentrations tested.

6. Quantitative analyses of free amino acids of the dogfish intestinal lumen showed variability in the absolute concentrations but great stability in the relative concentrations.

7. The data are discussed in terms of differences in amino acid entry systems of host and parasite, the significance of stability of amino acid ratios in the nutrition of host and parasite, and the necessity for evaluating host-parasite competitions in terms of entry of single components from complex mixtures.

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PIGMENTED FAT CELLS IN A MUTANT OF DROSOPHILA MELANOGASTER [1]

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The "red cell" mutant of *Drosophila melanogaster* is characterized by the presence of scattered pigmented cells in the thorax and head of the adult fly. This recessive mutant factor, *rc*, is located on the second chromosome, and histological examination of the *rc* phenotype by Jones and Lewis (1957) revealed that the red pigment was localized in granules in some of the pupal fat cells. The expression of the *rc* factor was suppressed in the presence of mutant genes which interrupt the synthesis of the brown pigment of the eye: *vermilion*, *scarlet*, and *cinnabar*. On the other hand, the mutant gene *brown* which blocks the synthesis of the red component of the normal eye color did not interfere with the expression of the *rc* gene as red cells in the thorax and head of the adult fly. Therefore Jones and Lewis (1957) concluded that the pigment in the red fat cells of *rc* flies is related to the brown pigment component of the eye.

This proposed relationship to insect eye pigments enhances the usefulness of the *rc* mutant as a tool in probing the pigmentation process at a cellular level. The large pupal fat cells offer excellent material for experimental manipulations of individual cells. In addition, the implantation experiments of Beadle (1937a, 1937b) with eye discs and various tissues of *Drosophila* established the fatbody as a source of pigment precursors for the synthesis of brown eye pigment. Preliminary observations of interaction between the *rc* mutant and a mutant strain which develops melanotic masses in the fatbody suggested the present study.

Materials and Methods

A stock homozygous for both the *rc* and tu^{w} factors was made, and all experimental procedures utilized material from this stock. The wild type strain, *Ore-R*, was used to verify the morphological relationships of the various fat masses in the larval stages. The *rc* mutant stock was kindly provided by Dr. E. B. Lewis in 1955, and the *rc* gene has been maintained in our laboratory in combination with the tu^{w} factor since that time. Melanotic tumorous masses occur in larvae homozygous for the recessive factor, tu^{w}, located on the second chromosome. Detailed studies of this mutant stock have been reported previously (Wilson *et al.*, 1955; Rizki, 1957).

The $tu^{w}rc$ stock has been raised on Cream of Wheat medium with Fleischmann's yeast. Timed material for the experiments was collected in the following manner. Adult flies were placed in a half-pint bottle containing a paper teaspoon with Cream of Wheat medium heavily coated with a yeast-honey suspension. A fresh

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food spoon was placed in the bottle twice daily, and after removal from the bottle the spoons were stored in an incubator at 23°–25° C. Beginning at approximately 20 hours after a spoon had first been placed in the collection bottle, the newly emerged larvae were collected at intervals of one or two hours. These larvae were raised on Cream of Wheat-Fleischmann's yeast medium in crystallizing dishes in the incubator at 23°–25° C. All ages of larvae and pupae are thus counted from the time of eclosion from the egg.

In the starvation experiments, larvae were removed from the food dishes at 65 hours of age, rinsed in a saturated solution of NaCl, 2% solution of NaOCl, followed by repeated washing in six changes of distilled water. This procedure removed a considerable proportion of the adhering yeast and food particles. Washed larvae were placed on tissue paper strips (Kleenex brand) moistened with distilled water in petri dishes; paper was also placed under the cover of the dish to prevent larvae from crawling out of the dishes. Care was taken to maintain the paper strip moist without excessive wetting. With each starvation experiment, a group of washed larvae from the same collection period was placed in petri dishes on paper strips to which a thin layer of Cream of Wheat medium and Fleischmann's yeast was added. These larvae served as the control fed material. The larvae removed from food at 65 hours of age generally pupated several hours before those which were left on food. No difference between the percentages of adults emerging in the two groups was noted.

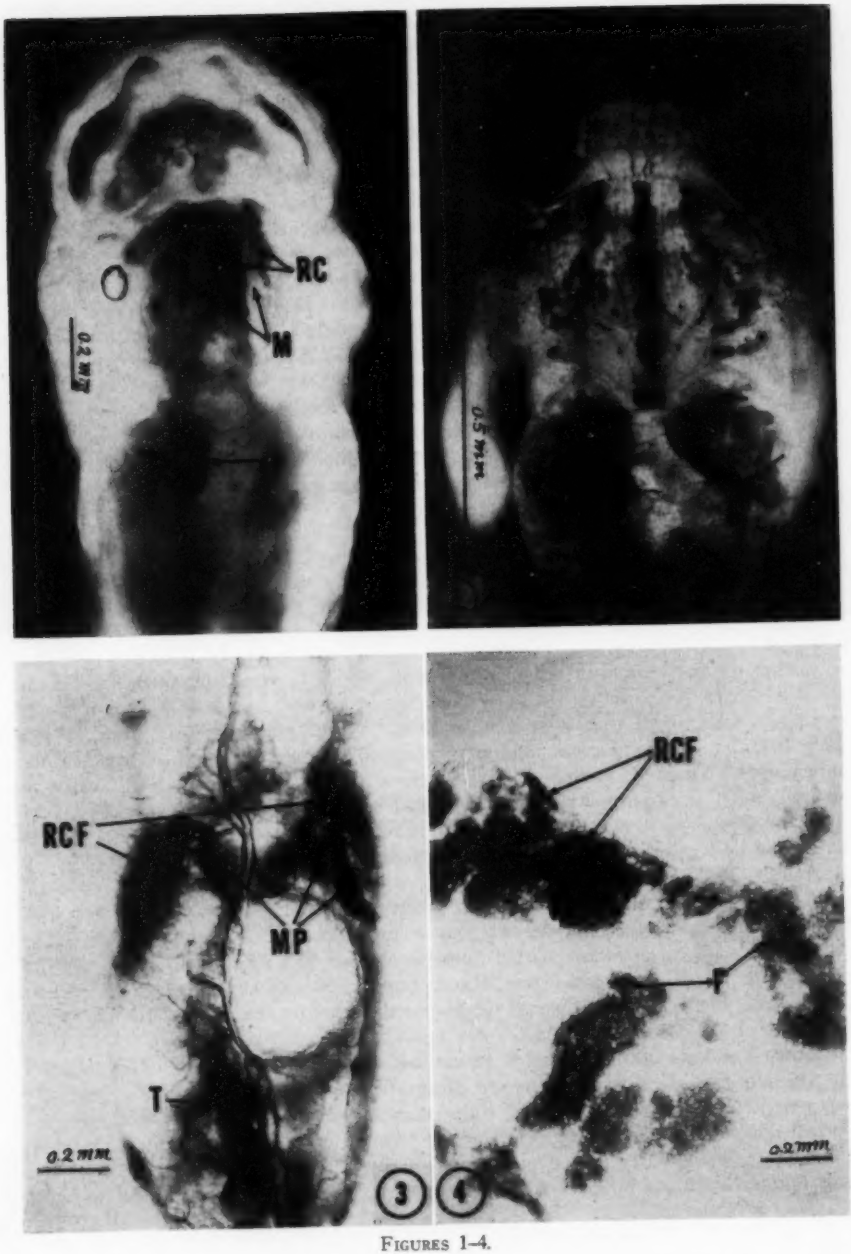
Results

No "red cells" appeared in the *tu*^{w}*rc* homozygotes in the stock bottles or in the control fed series. However, the expression of the *rc* factor was 100 per cent if the *tu*^{w}*rc* larvae were removed from food at approximately 65 hours of age, that is, during the early third instar. In this case, the mutant pattern appearing in the adult flies was the same as that described by Jones and Lewis (1957) for the *rc* stock. Red-pigmented cells were most abundant in the thorax and head, a few were found in the abdomen, and an occasional pigmented cell was seen in the appendages. In pupae shortly before hatching the red pattern in the thorax was striking. The red-pigmented cells occupied the haemocoel spaces between the flight muscles, and a dorsal striped pattern in the thorax was the result of alternating accumulations of red cells and regions of the muscle insertions (Figs. 1 and 2).

The *rc* factor exhibited no apparent influence on the development of melanotic masses in the posterior fatbody, the phenotypic character of the *tu*^{w} factor. The penetrance of the *tu*^{w} factor varied between 90% and 95% during the course of the present investigation; however, the *rc* phenotype was expressed in *tu*^{w}*rc* flies which had been starved during development whether melanotic tumors were visible or not.

Control fed larvae and starved larvae were examined at timed intervals after the beginning of the starvation period at age 65 hours. Starved larvae generally pupated two or three hours earlier than larvae which were feeding. No morphological differences were noted between the two groups of larvae until after pupation. During the early period of tanning of the puparium, a slightly yellow tinge was noticeable in the anteriormost fat masses of starved *tu*^{w}*rc* pupae. This color

PLATE I



FIGURES 1–4.

was visible through the puparium which was still very light, but removal of this covering was necessary to reveal the extent of the coloring. Figure 9 is a camera lucida drawing of a starved *tu*^{w}*rc* pupa showing the location of the yellow masses underneath the puparium, and a photograph of a specimen removed from the puparium shortly after the pupal molt is labelled Figure 3. Dissection of pupae showed that the yellow color was localized in the most anterior pair of dorsal fat masses just posterior to the cerebral hemisphere, as well as those fat masses which are lateral to this first region and extend ventrally where they join in a commissure. From the ventral side of each of these extensions, a strand of fat cells passes anteriorly where they adhere to the paired salivary glands. The fat masses just described are the only fat cells which developed the yellow pigment. Larvae of the *Ore-R* strain have been dissected to establish morphological correlations of the fat masses in the mutant strain with the fat masses in the normal material. Dissection of the larvae of the wild type strain was considerably easier since manipulation of the fat masses in the starved *tu*^{w}*rc* larvae tended to loosen the fat cells which did not seem to be held together so compactly as in the normal strain. Starvation also decreased the size of the fatbody cells as compared to fed material. In order to demonstrate the relationship of the intact fat masses, camera lucida drawings of the *Ore-R* strain are given in Figure 10. The common feature of these anterior fat masses is their proximity to the distal ends of the anterior pair of Malpighian tubules.

As pupation progressed the cells of the fatbody became separated from one another, and were thus involved in the extensive reorganization of the body tissues which takes place during metamorphosis. The pigmented cells of the anterior fatbody were redistributed during this process and were found primarily in the head and thorax of the developing pupa. The color of the fat cells in the intact fatbody of starved *tu*^{w}*rc* pupae gradually changed from yellow to yellow-brown, and as the scattering of the separated cells occurred, the color deepened, and finally red pigment was apparent. The yellow color in the fatbody appeared before the imaginal discs had everted, and the red pigment in the fat cells preceded the appearance of the red eye color.

FIGURE 1. Photomicrograph of a *tu*^{w}*rc* pupa (starved) removed from the puparium. Red cells, RC, are distributed between the longitudinal muscles, M, of the thorax. A typical melanotic tumor, T, is apparent in the abdomen and two *rc* fat cells are visible on the left margin of the tumor. (Darkfield illumination with a green filter. × 63.)

FIGURE 2. A fully formed imago removed from the puparium to show the further dispersion of the red-pigmented fat cells (arrows). Note the retention of *rc* cells in the mid-dorsal region and the further change in the distribution of *rc* cells in the areas not occupied by the insertions of the dorsoventral muscles of flight. Very few *rc* cells are seen among the fat cells of the abdominal region. The eyes are fully pigmented. (Darkfield illumination, green filter, magnification × 60.)

FIGURE 3. A *tu*^{w}*rc* (starved) prepupa removed from the pupal case, showing the position of the intact pigmented masses of fat, RCF. Note the intimate association of the anterior pair of Malpighian tubes, MP, with the RCF. The photograph represents the left dorsolateral view of the prepupa so that the left tracheal trunk is apparent and the right tracheal trunk can be visualized as a white band at the lower right margin of the photograph. The dark area under the left tracheal trunk is a melanotic tumor in the caudal fatbody, T. (Transmitted light from a Corning filter #CS 7-59. Magnification × 63.)

FIGURE 4. The intact fat masses removed from a *tu*^{w}*rc* prepupa (starved) showing the difference between pigmented fat masses, RCF, and unpigmented fat masses, F. This fat was treated with potassium metabisulfite to intensify the color. (Corning filter #CS 7-59; × 63.)

PLATE II

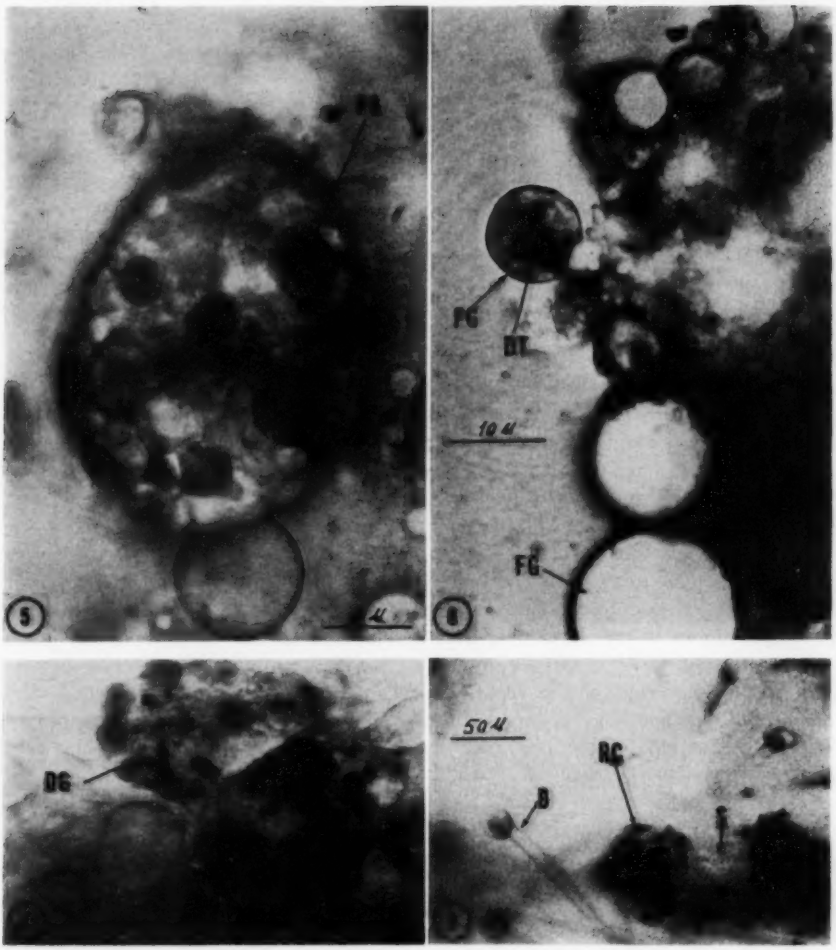


FIGURE 5. A pigmented fat cell isolated from an early prepupa (*tu*^{w}*rc* starved) showing the intracellular distribution of pigment globules, PG. (Corning filter #CS 7-59.)

FIGURE 6. A pigment globule removed from the cytoplasm of a red cell from a *tu*^{w}*rc* prepupa (starved) showing the threadlike, DT, internal structures characteristic of these globules. A fat droplet is indicated at FG. (Corning filter #CS 7-59.)

FIGURE 7. The *rc* fat cells as seen through the body wall of a *tu*^{w}*rc* starved pupa corresponding in age to that given in Figure 1. Note the appearance of pigment in granular form. (Darkfield illumination, green filter.)

FIGURE 8. Granular appearance of pigmented inclusions in a *tu*^{w}*rc* starved imago corresponding in age to that in Figure 2. Bristles, B, and setae, S, are visible in this photograph of a red cell as seen through the body wall. (Darkfield illumination, green filter.)

Both the red and brown pigment extracts from the eye of *Drosophila* are altered by oxidation and reduction (Ephrussi, 1942). It seemed desirable to determine whether the yellow pigment in the fat cells would undergo any changes *in vitro*, and a search was undertaken for conditions which might cause such an alteration. Anterior fat masses which had become yellow were dissected from starved pupae in Waddington Ringer-10% glucose. The various reagents to be tested were then added to this medium. The reducing rinse which had been prepared for use in the Feulgen reaction proved most satisfactory. It was then found

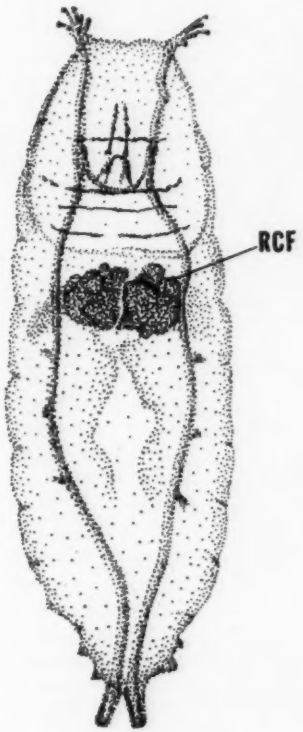


FIGURE 9. Camera lucida drawing of a dorsal view of a *tu*^{w}*rc* (starved) prepupa within the puparium illustrating the position of the brownish yellow anterior fat mass, RCF.

convenient to add a few crystals of potassium metabisulfite to the drop of glucose-Ringer containing the isolated fat masses while they were under microscopic observation on a white porcelain plate. The color of the fat cells showed a change from yellow to red within a minute. Figure 4 is a photograph of the isolated fat mass in which the *rc* pigment had been intensified in this manner.

The development of pigment in the starved *tu*^{w}*rc* flies has been followed at the cellular level. Isolated cells from the anterior fatbodies of young pupae contained

numerous yellow cytoplasmic globules. These globules were distinguishable from the fat droplets of the cells which are highly refractile and always spherical in fresh preparations. Isolated cells have been examined with darkfield illumination as well as brightfield illumination, and in addition, the use of a blue Corning

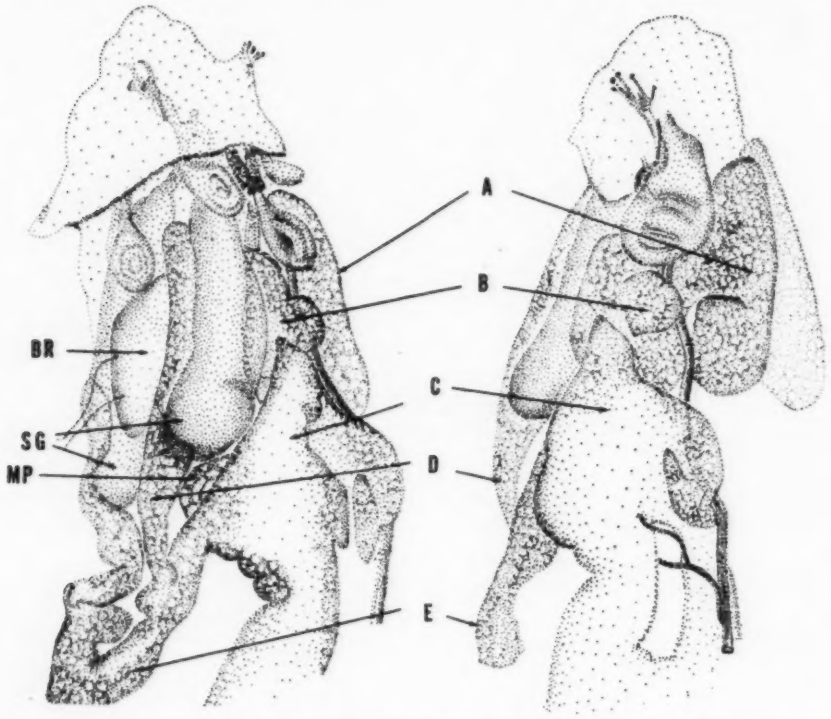


FIGURE 10. Camera lucida drawings of a dissection of a late third instar larva of the *Ore-R* strain showing the relative positions of the fat masses; the salivary glands, SG; brain, BR; Malpighian tubes, MP. This specimen has been stained with Oil Red O. The diagram on the left is the ventrolateral view, and the same dissection has been turned over to show the lateral view of the fat masses in the drawing on the right. A, B, C, D, E are the regions of the fatbody which become pigmented in starved $tu^{w}rc$ pupae. A, dorsal pair of fat masses; B, C, lateral fat masses; D, fat mass attached to salivary gland; E, fat cells forming the ventral commissure between the right and left masses of fat. Only the anterior region of C becomes pigmented. The club-shaped structures with concentric rings are the imaginal discs and the long tubular structure with branches is the tracheal trunk.

Filter No. CS 7-59 proved most satisfactory for studying the cytoplasmic inclusions in the fat cells. With this filter, all yellow objects appeared bright red. In early $tu^{w}rc$ pupae (after starvation), this included the lightly tanned cuticle, isolated cells from melanotic masses, the granular structures in the Malpighian tubules,

and the yellow globules in the *rc* fat cells. The yellow globules showed a definite threadlike internal structure when examined with this filter, while no structure was discernible in the fat droplets (Figs. 5 and 6). Fat cells from the more posterior regions of the pupa, *i.e.*, cells other than the fatbody cells involved in the expression of the *rc* phenotype, contained similar cytoplasmic inclusions in addition to the fat droplets. Whether these inclusions are structurally and functionally the same as those globules which become pigmented in the *rc* cells remains to be examined. The cells containing the yellow globules were placed on a slide in Waddington-10% glucose solution and several crystals of potassium metabisulfite were added under the coverslip in the vicinity of the cells. The yellow inclusions became red in color under these conditions.

After the pigmented fat cells had become scattered throughout the thorax and head during the developmental processes occurring in pupal life, the color in the fat cells appeared more intense. In the late pupae and young adults, the pigmented structures in these cells were more granular in appearance (Figs. 7 and 8).

Discussion

Two types of pigment are found in the eye of *Drosophila*, one brown and the other red, and many mutants are known which affect the production of these pigment components in the eye. Interference with brown pigment production results in a bright red eye color of the type found in the mutants *vermilion*, *cinnabar*, and *scarlet* while the phenotype of the *brown* eye mutant represents an interruption in the biochemical pathways leading to red pigment. The absence of both pigments occurs in the mutant, *white* eye. The literature on brown eye pigments in insects has been reviewed by Ephrussi (1942) Nolte (1952), and Kikkawa (1953). The synthesis of brown pigment proceeds through a pathway involving tryptophan, formylkynurenine, kynurenine, and hydroxykynurenine, and the known eye color mutants are blocks at successive stages in this synthetic chain. Transplantation of imaginal discs of mutant larvae into hosts of different genotypes has shown that in some cases the eye color is autonomous, whereas some mutant eyes do not themselves produce the prerequisites for brown pigment and are dependent upon other sources in the body for these precursors (Beadle and Ephrussi, 1936; Ephrussi, 1942; Ephrussi and Beadle, 1937). The presence of these pigment precursors has been demonstrated in the fatbody and the Malpighian tubules by transplantation experiments (Beadle, 1937a, 1937b). The time during which each of these tissues produced the pigment precursors was dependent upon the stage of development of the donor. Malpighian tubes showed activity through larval life from the earliest stages tested, appearance of active substances in the fatbody was not detected until after pupation, and in the eyes much later during pupal development (Beadle, 1937b; Clancy, 1940).

The pigment granules in the *rc* cells have been shown to be related to the brown eye pigment of *Drosophila*. Jones and Lewis (1957) found that the mutant factors, *vermilion*, *cinnabar* and *scarlet*, which interfere with brown pigment development in the eye, also prevent the formation of pigment in the *rc* cells when each of these mutant factors is combined with the *rc* gene. The mutant factor, *brown*, which blocks the synthesis of red pigment, does not interfere with the expression of the *rc* gene in the fat cells.

The explanation for the suppression of pigment in the fat cells when the *rc* gene is combined with the *tu*^{w} factor may not be so direct. In the starvation experiments the penetrance of the *tu*^{w} factor was 90%–95%, and no difference in the expression of *rc* was noted between the pupae with melanotic tumors and those that did not develop black masses. One point of comparison is the fact that both mutants have a common domain of expression, *i.e.*, fat cells: *rc*, the anterior fat mass and *tu*^{w}, the posterior region of the fatbody. Tryptophan metabolism is not only related to the development of brown eye pigment and protein metabolism, but it also influences the expressivity and the penetrance of various melanotic tumor genes in *Drosophila*. Addition of tryptophan to the medium increases the frequency of melanotic tumors in strains carrying tumor genes (Hartung and Hartnett, 1951; Plaine and Glass, 1955), and Kanehisa (1956a, 1956b) reported an increase in tumor incidence by combining a tumorous factor with eye color genes. The appearance of pigmented fat cells in *tu*^{w}*rc* pupae after starvation parallels the behavior of the *vermilion* mutants which develop brown eye pigment after the larvae have been starved (Beadle *et al.*, 1938). Starvation of *Drosophila* adults results in a reduction in the size of the fatbody, and the reserves of fat and glycogen are rapidly depleted from the fat cells (Wigglesworth, 1949). In many insects the fatbodies may serve as storage sites for excretory products as well as food reserves. Wigglesworth (1942) has shown that starvation of *Aëdes* larvae causes an increase in uric acid vacuoles in the fat cells and these deposits disappear from the cells after the feeding has resumed. The conditions imposed by starvation in the *tu*^{w}*rc* larvae alter the metabolic pattern of the fat cell such that it differentiates as a pigmented cell. A similar effect, of course, is produced in the *rc* mutant under normal feeding conditions. The presence of the *tu*^{w} factor may restore the normal metabolic balance in the fat cell such that their phenotype resembles that of the wild type. The expression of the *rc* phenotype is also dependent upon the action of another recessive gene, *lys*, which causes an accumulation of the amino acid, lysine (Grell, 1958). It is thus obvious that the expression of the red cell phenotype is influenced by the interaction of a number of non-allelic genes. One suggestion may be made which will encompass the various aspects of the problem known at the present time. Any modification, genetic or environmental, which influences the normal pattern of protein synthesis will also alter the metabolic pool of various amino acids. Such changes which affect the availability of tryptophan may be reflected in the phenotypic expression of the *rc* pigment.

The larval fat cells of *Drosophila* form organized tissue masses, whereas soon after pupation the fatbody becomes separated into single cells or small clusters of cells. The cells of the caudal fat masses in *tu*^{w} larvae which are involved in the production of melanotic tumors in this strain resemble pupal fat cells in their tendency toward smaller cell aggregations and a loss of adherence to neighboring cells (Rizki, 1957). This precocious change in the structure of the caudal fatbody of *tu*^{w} larvae, as well as precocious changes in the blood cells, are processes which lead to tumor formation in the caudal fatbody prior to pupation. Therefore the hypothesis was presented that the melanotic masses in this tumorous strain of *Drosophila* represented an upset in the normal timed pattern of events occurring during metamorphosis. Under conditions favoring expression of the *rc* gene, the

tu^{w} factor influences an earlier appearance of red pigment in the anterior fat cells. This pigmentation in the *rc* mutant is not apparent until after the fat cells become isolated and scattered during the pupal stage (Jones and Lewis, 1957). However, the combination of *rc* with the *tu*^{w} factor has shifted the time of development of the red pigment to a stage preceding this dispersion of the fat cells. Although no obvious explanation for the distribution of the red fat cells among other nonpigmented fat cells existed in the *rc* strain, the morphological relationship of all the red cells in larval development becomes apparent in the *tu*^{w}*rc* starved material. A measure of the dispersion of the cells of the anterior fat masses during early pupation is provided in this case by a mutant cytoplasmic marker. The localization of mutant characteristics in the *tu*^{w} caudal fat masses and the anterior *rc* fat cells suggests that the cells of various regions of the fatbody may differ in their developmental physiology. It is interesting to note that the cellular ecology of the anterior and the posterior fatbody includes a common feature: the *rc* fat cells are intimately associated with the distal ends of the anterior pair of Malpighian tubules, and the *tu*^{w} fat cells encircle the distal ends of the posterior Malpighian tubules.

Summary

1. Cytoplasmic pigment granules are found in some of the fat cells of the recessive mutant, *rc*, of *Drosophila melanogaster*. These scattered red fat cells are located chiefly in the thorax and head, but a few occur in the abdomen and appendages of the adult. Using genetic methods it had been shown previously that these pigment accumulations are related to the synthesis of the brown eye pigment of this insect (Jones and Lewis, 1957).

2. The pigmentation in the *rc* fat cells is suppressed when the *rc* gene is combined with the recessive factor, *tu*^{w}. This combination, however, in no way alters the expression of the characteristic pattern of *tu*^{w} as revealed by the presence of melanotic tumors in the caudal fat masses of the homozygous *tu*^{w}*rc* flies. After a period of larval starvation, the *tu*^{w}*rc* flies develop both the red-pigmented fat cells and melanotic tumors. The time of appearance of the *rc* pigment has been shifted under these nutritional and genetic conditions. The cytoplasmic pigment granules appear in the cells originating from the anteriormost section of the larval fatbody which is closely associated with the anterior Malpighian tubules. During the reorganization accompanying metamorphosis from the larval to the adult stage, these cells are redistributed mostly to the thoracic and cephalic regions while a few are found in the abdomen and appendages. An explanation is thus provided for the cytodifferentiation of pigmented and nonpigmented fat cells found side by side in the adult fly.

3. The nature of the pigment granules has been examined in *in vitro* preparations at each of these periods of development, and of particular interest is the internal threadlike structure of these cytoplasmic inclusions during the early stages of pigment formation.

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EXPERIMENTAL STIMULATION OF GAMETOGENESIS IN HYDROIDES DIANTHUS AND PECTEN IRRADIANS DURING THE WINTER[1]

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There is a long list of benthic marine invertebrates found in the Woods Hole, Mass. region that reproduce during the summer months. The majority of the species of organisms suitable for embryological experiments, listed by Costello *et al.* (1957), fall into this category. In addition, the extensive investigations of Redfield and Deevey (1952) have shown that most members of the fouling community attach during the summer months at Woods Hole and in other localities where there is a considerable difference between the summer and winter temperature extremes.

The question arises as to which of the various environmental variables controls reproduction. Hutchins (1947) studied the world-wide distribution of a variety of benthic marine forms and came to the conclusion that the northward extension of the ranges of a number of organisms is regulated by the minimal summer temperature that permits propagation, thus suggesting that elevated temperatures either stimulate gametogenesis or induce spawning.

Experimentally, Townsend (1940) obtained ripe gametes from the sea urchin *Arbacia* by holding specimens in aquaria at a temperature of 18° to 19° C. for one to two months in the late fall and winter. An ample supply of food was provided. Subsequently, Loosanoff and his co-workers have contributed greatly to the field by demonstrating the stimulating effects of elevated temperatures on the development of the gonads of certain commercial mollusks. Loosanoff and Davis (1950) succeeded in bringing the hard clam, *Venus mercenaria*, into reproducing condition during the winter months by gradually raising the temperature over a period of three weeks from that of the natural environment (5.0 to 7.0° C.) to 20° C. They mentioned that the same could be accomplished by placing the clams directly in water at 20° C. but some mortality occurred. Later (1952) these investigators studied the influence of temperature on the maturation of the gonad of the eastern oyster, *Crassostrea virginica*, and demonstrated a logarithmic increase in the rate of ripening of the gametes as temperatures were elevated from 15° C. to 30° C. They also determined that physiologically ripe gametes were formed earlier in the males than in the females under the same conditions. The importance of nutrition on gonad development was indicated by the failure of oysters in "poor" condition, *i.e.*, containing little glycogen, to respond satisfactorily to the thermal stimulus.

Experimental work on the effect of temperature on gonad development of

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other benthic marine invertebrates appears to be lacking. The investigations to be described were undertaken to determine if certain organisms occupying approximately the same geographical range as that of the oyster and the hard clam would respond in a similar fashion. The serpulid polychaete, *Hydroides dianthus* (= *H. hexagonus*) (Verrill), ranges from Cape Cod to Florida (Pratt, 1948) and is found from the low tide mark to depths of several fathoms. It reproduces from the middle of June to the end of October (Grave, 1933) and the ripeness of the gametes may be easily determined by removing the worms from their tubes and observing the products issuing from the nephridiopores (Grave, 1933). The bay scallop, *Pecten irradians* Lamarck, ranges from Cape Cod to Texas (Turner, 1953). It is a hermaphroditic species that spawns from mid-June to mid-August in the Woods Hole region (Belding, 1931). The ripening of the reproductive products may be determined on macroscopic examination by the development of a bright orange color in the ovary at the distal extremity of the visceral mass.

Material and Methods

Specimens of *H. dianthus* were obtained from the supply department of the Marine Biological Laboratory in the middle of December. They had been freshly collected from the adjacent waters where the temperature was approximately 8° C. The specimens were attached to old *Venus* and *Pecten* shells in a community which included *Astrangia*, sulphur sponges and dead barnacle shells. The tubes ranged between 5 and 7 cm. in length, well beyond the size of earliest sexual maturity (Grave, 1933).

Bay scallops were collected from the Eel Pond, Woods Hole, in December and January, in shallow water at low tide. They averaged a little over 3 cm. in the longest dimension and were the young of the year (Belding, 1931). There were no year-and-one-half old specimens in the Eel Pond and those usually found in nearby localities had been almost completely depleted by commercial fishing. Consequently, larger specimens were not available in adequate numbers for experimental work.

At the beginning of the experiments, a number of specimens of *H. dianthus* were removed from their tubes and placed in warm (23° C.) sea water to determine if they would extrude ripe gametes. No gametes were extruded and the worms were preserved in saturated aqueous mercuric chloride with 5% acetic acid for histological study. Similarly a number of scallops were opened and examined for the characteristic orange color of the ripe ovary. None showed this character and a number of appropriate anatomical portions were preserved in the same manner as were the worms.

The worms were subjected to an elevated temperature in aerated still water. Several tube-encrusted shells, containing about two dozen worms, were placed in a non-toxic five-gallon polyethylene container of warmed sea water held at 23° C. by a constant temperature bath. The water was changed every three days and continuously aerated. Suspensions of the planktonic alga, *Phaeodactylum tricornutum* (= *Nitzschia closterium, forma minutissima*), were added daily for food. The amount added was adjusted to the quantity that the worms would just consume in 24 hours. A control was set up and maintained in an identical manner except that the container was placed in a tank supplied by the laboratory sea water

system in which the temperature declined slowly from an initial level of 8° C. The illumination of the experimental group and the control group was practically identical. Growth of the tubes was determined in most cases by measuring the new white addition at the mouth beyond the discolored and fouled original tubes. In cases where the tubes were not sufficiently discolored to make new growth clearly distinguishable, a gram of powdered alizarin was added to the sea water and kept in suspension for 24 hours. The worms readily took up the alizarin and combined it with the calcareous material of the new growth, forming a prominent purple ring of alizarin lake. This treatment provided a very satisfactory method of marking the tubes and apparently had no deleterious effect on the worms. Specimens were removed on the third, fifth, seventh, and tenth days, tested for extrusion of gametes and preserved for histological examination. The experiment was repeated four times during December, January and early February.

Preliminary experiments indicated that bay scallops could not be maintained successfully in still water so they were treated in a different manner. In late January a number of specimens were placed in a $3' \times 3' \times 8''$ Fiberglas tank supplied with running warmed sea water at a rate of $\frac{1}{2}$ gallon per minute. The apparatus used to warm the water was similar to that described by Loosanoff (1949) but modified to use electric power instead of gas. The temperature was maintained at 23° C. No food was added because the laboratory facilities for rearing phytoplankton were inadequate to supply the vast quantities required in running sea water systems. Consequently the scallops had to subsist on such quantities of food materials as remained in the water after it had passed through the intake line, reserve tank, and lengthy distributing system. A control was set up in a similar manner except that it was supplied with sea water at the ambient temperature which was holding steady at approximately 3° C. The lighting conditions over both groups were identical. Specimens were removed weekly, examined grossly for gonad development and preserved for sectioning.

Results

Hydroides dianthus

None of the worms shed gametes when first obtained, nor did any taken from the control group which was maintained at a temperature approximating that of the natural environment at any subsequent time. In the experimental group which was maintained at 23° C., gametes were first obtained after seven days of warming. Males shed copious quantities of reproductive material consisting largely of spermatocytes and spermatids with a few tailed spermatozoa. By this time, females shed numerous eggs which were very much smaller than normal size and could not be fertilized. On the tenth day all males tested produced normal spermatozoa and the females shed eggs, the majority of which were of normal size. These eggs were successfully fertilized, underwent normal cleavage according to the usual time schedule (Costello *et al.*, 1957), and produced vigorous trochophores. During the ten days of warming all the worms increased the length of their tubes from three to five millimeters, indicating that they were vigorous and healthy. Worms in the control group showed no measurable growth.

The sequence of events during gametogenesis of the tube worm was followed

in histological sections. The winter gonad consists of a series of pairs of syncytial masses of germ cells, one pair to each of the abdominal segments. Each segment contains a mass of reproductive material on either side at the ventro-lateral aspect of the wall of the coelom. Figure 1, A is a typical cross-section of a worm pre-

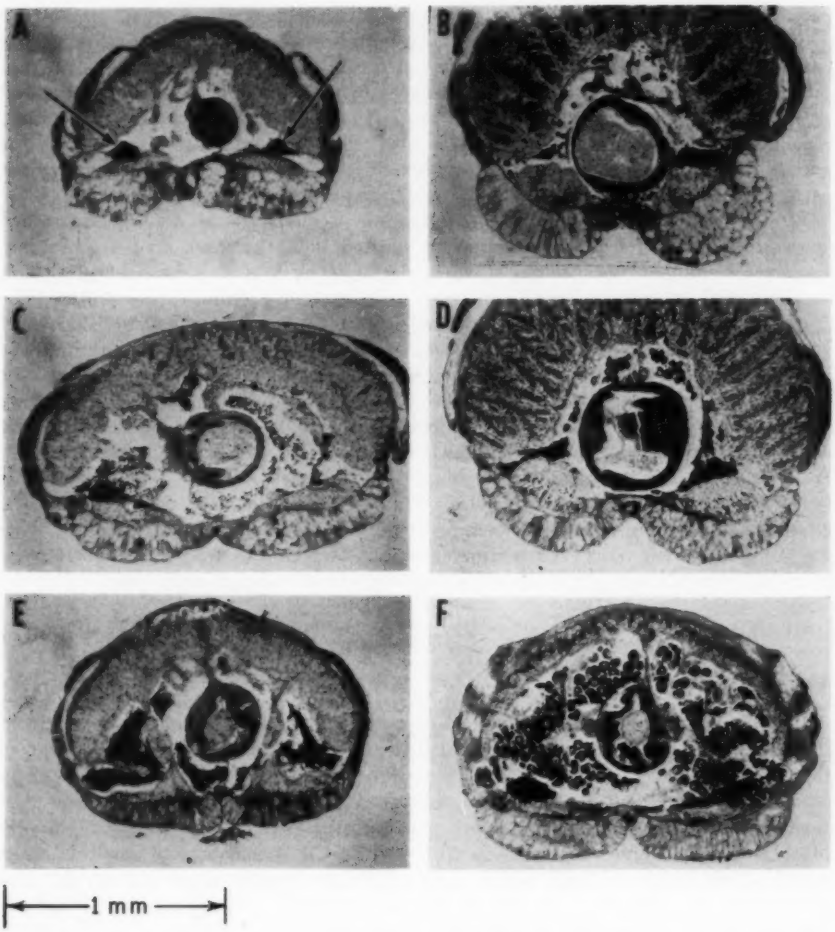

FIGURE 1. Transverse sections of *H. dianthus*. Delafield's haematoxylin and eosin stain. A, Specimen collected in December. Germinal masses indicated by arrows. B, Female held at 23° C. for five days. A few ovocytes free in the coelom. C, Male held at 23° C. for three days. Numerous spermatocytes free in the coelom. D, Female held at 23° C. for seven days. Numerous ovocytes partially grown free in the coelom. E, Male held at 23° C. for ten days. Mature spermatozoa in the coelom. F, Female held at 23° C. for ten days. Mature ova in the coelom.

served in mid-winter. There was little evidence of sexual differentiation at this time although some specimens contained masses with slightly enlarged nuclei, suggestive of primitive ovocytes. All specimens examined contained a few mitotic figures in the germinal masses, indicating that the germinal material was proliferating slowly.

Sexual differentiation became apparent in males after three days of warming. Cells broke away from the germinal masses into the coelom where the maturation divisions took place (Fig. 1, C). This process continued for the next seven days and by the tenth day the coelom was packed with mature spermatozoa, as shown in Figure 1, E.

No significant changes in the female gonads were observed until the fifth day of warming. At this time the nuclei began to enlarge as typical germinal vesicles and a few ovocytes broke free into the coelom (Fig. 1, B). By the seventh day, there were more free ovocytes in the coelom and some had shown appreciable growth (Fig. 1, D). By the tenth day, the coelom was completely filled with ovocytes, the majority of which were of mature size (Fig. 1, F).

There was remarkable uniformity in the rate of development of the gonads in all specimens examined and this was repeated in each of the four experiments run in sequence. At no time did any of the animals taken from the low temperature controls show any evidence of gametogenesis.

Pecten irradians

The reaction of the bay scallops to the elevated temperature was slower and more erratic than that of the tube worm. Approximately 5% died during the course of the experiment. A few specimens failed to show any development of the gametes, and in many, gametogenesis proceeded for a short time and then ceased. The majority of the specimens examined eventually produced tailed spermatozoa and ova which appeared to be structurally mature on histological examination. However, normal spawning did not occur so that physiological maturity could not be determined.

The bay scallop is hermaphroditic. The gonad consists of cylindrical, branching, tubular follicles ramifying through the visceral mass in the blood space surrounding the digestive tract. The follicle wall consists of a single layer of squamous epithelium with germ cells scattered along the inside. Male and female germ cells develop in separate follicles.

Sections of specimens taken from the natural environment in January showed no significant gametogenesis. The follicles of the male gonad as they appeared in sections are shown in Figure 2, A. The follicle walls of the female gonad (Fig. 2, B) appeared to be thicker because of the larger sizes of the ovogonia but there was no evidence of significant development.

Marked changes occurred after the scallops had been subjected to a temperature of 23° C. for one week. Rapid proliferation of the germinal material in the male resulted in a multi-layered lining of the follicles, consisting of spermatogonia and spermatocytes (Fig. 2, C), while the germ cells of the female gonad (Fig. 2, D) enlarged considerably and develop characteristic germinal vesicles with prominent nucleoli (Fig. 2, D.)

At the end of the third week of warming, the ovarian portion of the gonad acquired the orange pigmentation characteristic of the ripe ovary and the testicular portion took on a light, cream color with a plump appearance. Histological sections showed that the male follicles were lined with many layers of spermatozoa with the tails projecting out into the much reduced lumens (Fig. 2, E). The ovarian follicles were much enlarged and were completely filled with ova of mature size

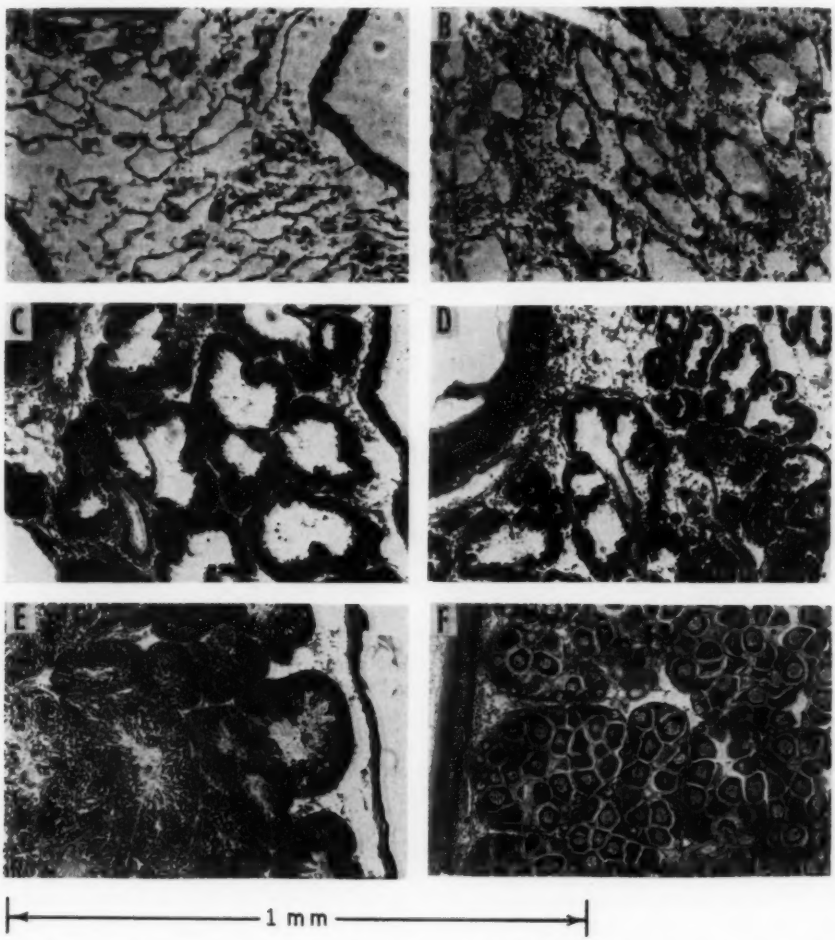

FIGURE 2. Sections of gonads of *P. irradians*. Delafield's haematoxylin and eosin stain. A, Male gonad of specimen collected in January. B, Female gonad of same specimen. C, Male gonad of specimen held at 23° C. for one week. D, Female gonad of the same specimen. E, Male gonad of specimen held at 23° C. for three weeks. F, Female gonad of same specimen.

(Fig. 2, F). None of the specimens taken from the control group showed any evidence of gametogenesis during January and February.

Discussion

It is clear from the foregoing that the tube worm, *Hydroides dianthus*, and the bay scallop, *Pecten irradians*, respond to artificially elevated temperatures during the winter in a manner similar to that shown by many commercial molluscs, as described by Loosanoff, by developing their gametes out of season. All of the tube worms subjected to a temperature approximating that of the natural environment during the normal reproductive period developed ripe gametes within ten days. These underwent normal fertilization, cleavage, and developed into viable larvae.

The response of the bay scallop was somewhat erratic and the development of the gametes was much slower. It is quite probable that the experimental conditions were not entirely satisfactory, particularly in regard to nutrition. Oysters and clams maintained in the laboratory sea water system grow thin and watery after a time, indicating that suspended nutrient material is sparse. Consequently, the scallops held in running sea water at the elevated temperature undoubtedly had to synthesize the reproductive materials from stored substances which may have varied considerably among individuals at the time they were collected. Individuals in which gametogenesis failed to go on to completion were probably those with inadequate supplies of reserve nutrients, similar to the oysters in "poor" condition described by Loosanoff and Davis (1952). In any event it is clear that temperatures approximating those existing during the normal spawning period will stimulate gametogenesis in both *P. irradians* and *H. dianthus* if imposed experimentally during the winter months when the temperature of the natural environment is approaching the seasonal minimum.

Summary

1. Temperatures approximating those existing during the normal summer reproductive period will stimulate gametogenesis in *Hydroides dianthus* and *Pecten irradians* if artificially imposed during the coldest winter months.

2. *H. dianthus* will produce mature gametes in ten days if held in aerated sea water at 23° C. and fed adequate quantities of *Phaeodactyllum tricornutum*.

3. *P. irradians* may produce gametes that appear to be mature on histological examination in three weeks if held in running sea water warmed to 23° C. but in many cases development fails to go to completion.

4. Failure of gametogenesis to reach completion in some specimens of *P. irradians* may be due to inadequate food supply under the conditions of the experiment.

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A NEW SPECIES OF CHIRIDOTEA (CRUSTACEA: ISOPODA) FROM NEW ENGLAND WATERS

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The genus *Chiridotea* is unique to the eastern coastal region of the United States and southeastern Canada. Members of this genus are small (usually < 1 cm.), broad, depressed valviferous forms that inhabit sandy areas and characteristically burrow just beneath the sediment surface. The species described herein constitutes the fourth known species of this genus. Those previously described are: *C. coeca* (Say, 1818); *C. tuftsi* (Stimpson, 1853); and *C. almyra* Bowman, 1955. Included in Bowman's paper is a revision of the generic characteristics of this group. The description of this new form is based on specimens collected on Georges Bank, a relatively shallow portion of the continental shelf east of Massachusetts, U. S. A. These specimens were encountered while processing collections of benthic invertebrates taken by the R/V *Albatross III* and the R/V *Delaware* for the Woods Hole Laboratory, Bureau of Commercial Fisheries, U. S. Fish and Wildlife Service.

Material Examined.—Holotype, adult female with oostegites developed, 7.5 mm. in length, deposited in the U. S. National Museum (Catalogue No. 104282); allotype, adult male 6.0 mm. in length (U.S.N.M. Catalogue No. 104280); paratypes, 1 male 5.0 mm. in length and 1 ovigerous female 6.5 mm. in length (U.S.N.M. Catalogue No. 104281); all type specimens were collected August 6, 1959, by means of a grab-type bottom sampler on Georges Bank at lat. 41° 48′ N., long. 67° 53′ W.; water depth 15 fathoms, sand substrate, bottom water temperature 57.0° F. (R/V *Delaware*, cruise number 59-9, station 21).

One female, 7.5 mm. in length, collected December 7, 1955, at lat. 40° 51′ N., long. 68° 55′ W.; substrate coarse sand, water depth 36 fathoms, bottom water temperature 47.2° F. (R/V *Albatross III*, cruise number 70, collection 3).

One male, 6.5 mm. in length, collected December 14, 1955, at lat. 41° 40′ N., long. 67° 36′ W.; substrate gravelly sand, water depth 28 fathoms, bottom water temperature 45.7° F. (R/V *Albatross III*, cruise number 70, collection 38).

Five females, body lengths 4.0, 5.0, 6.5, 7.5, 7.5 mm., and two males, body lengths 7.0 and 7.0, collected August 24, 1957, at lat. 41° 22′ N., long. 68° 20′ W.; substrate coarse sand, water depth 24 fathoms (R/V *Albatross III*, cruise number 101, station 64).

Three specimens, 4.0, 4.5, 4.5 mm. in length, collected August 24, 1957, at lat. 41° 34′ N., long., 67° 28′ W.; substrate coarse sand, water depth 23 fathoms (R/V *Albatross III*, cruise number 101, station 90).

Diagnosis.—Medium-sized *Chiridotea* with short antennae; flagellum of antenna 2 is much shorter than the peduncle; antenna 1 usually not reaching beyond the peduncle of antenna 2; outer margin of pereion epimeral plates 5–7

FIGURES 1–9. *Chiridotea arenicola* n. sp.

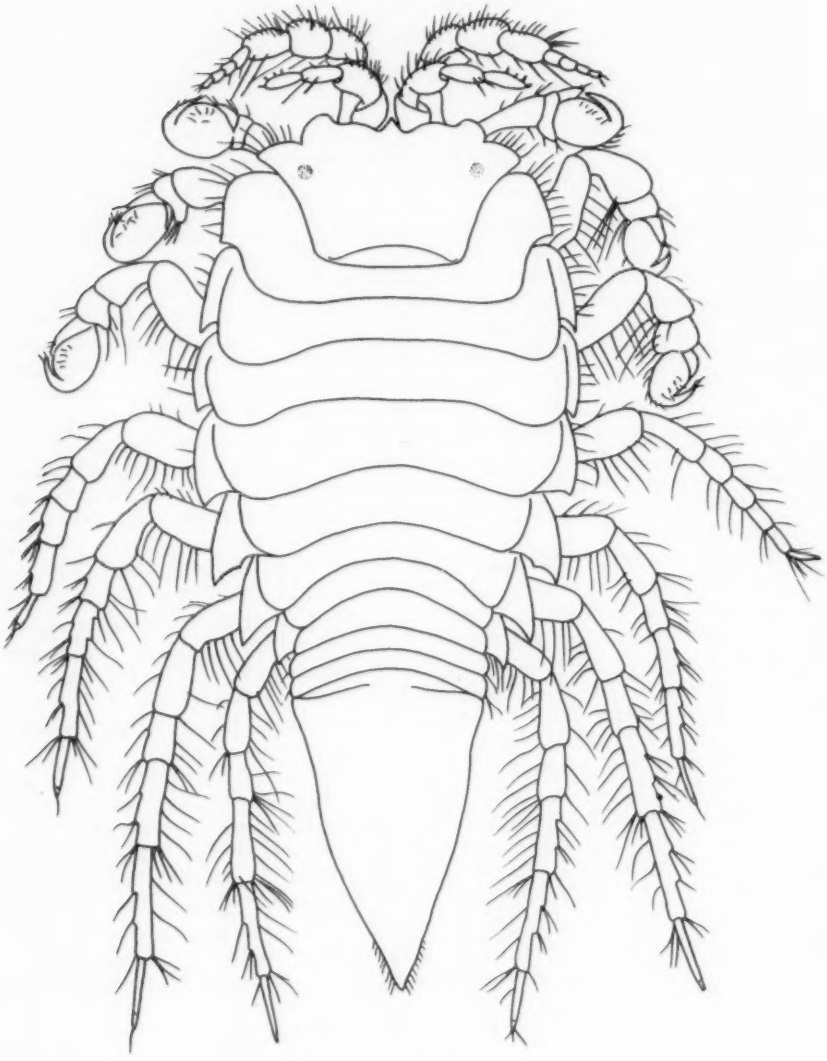

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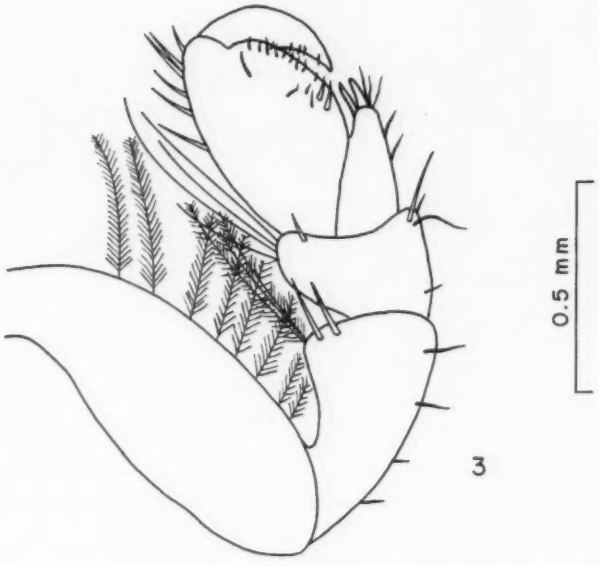
FIGURE 1. Dorsal view of female holotype.



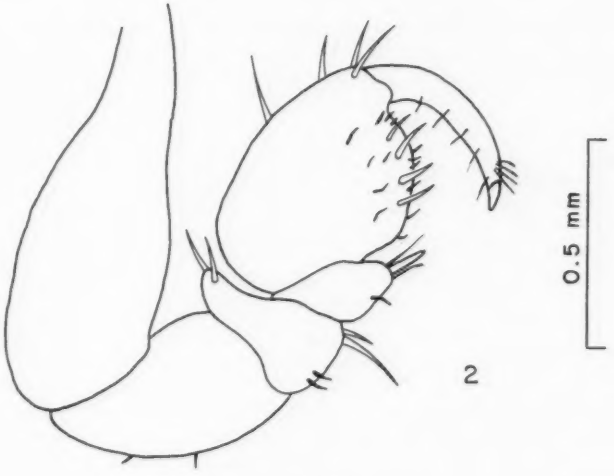


FIGURE 2. Pereiopod 1, ♂.
FIGURE 3. Pereiopod 2, ♂.

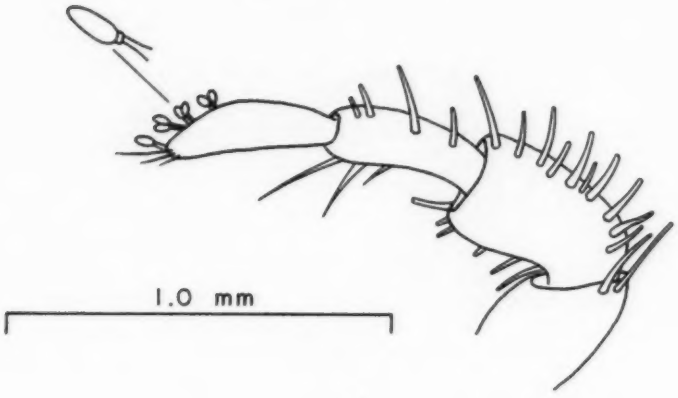


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FIGURE 4. Antenna 1, ♂.

with relatively few or no setae. This species most closely resembles *C. coeca* (Say) but differs in that it is smaller, the pereiopods are more slender, the body is thinner and less convex dorso-ventrally, the pleotelson is narrower and more evenly tapered, and the anterior lobes of the antero-lateral sections of the head are shorter and have margins devoid of setae. Other differences are mentioned later in this paper under the heading *Discussion*.

Description.—Anterior margin of head broadly and shallowly excavated each side of the rostrum. Lateral anterior projections on the head are rounded or obtusely pointed. Pronounced, but comparatively shallow, V-shaped notch in each antero-lateral margin of the head; margin anterior to the notch is without

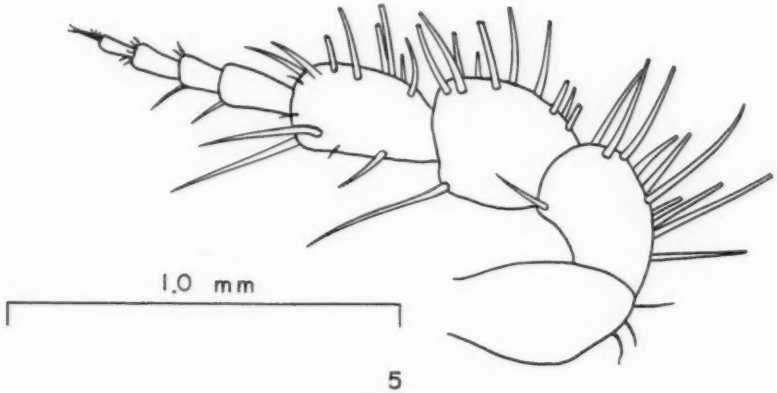

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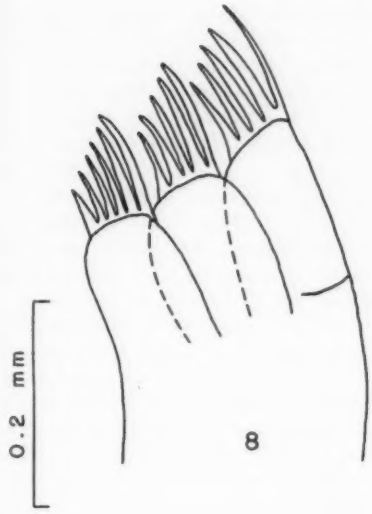
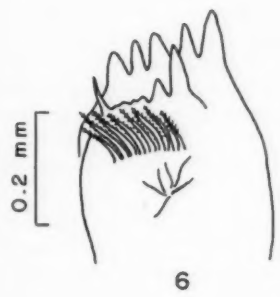
FIGURE 5. Antenna 2, ♂.





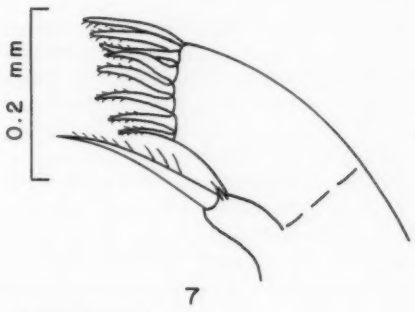


FIGURE 6. Left mandible, ♂.
FIGURE 7. Maxilla 1, ♂.
FIGURE 8. Maxilla 2, ♂.
FIGURE 9. Maxilliped, ♂.

setae, the margin posterior to the notch has approximately 6 setae. The anterior lobe does not extend outwardly as far as the posterior lobe. Rather small, irregularly rounded compound eyes are located dorsally on the head near the base of the postero-lateral lobe; they are only faintly visible in many of the specimens examined. Epimeral plates distinct on pereion somites 2–7, their postero-lateral margins acutely produced posteriorly; this projection is more pronounced

on plates 5–7 than on plates 2–4. Stout setae fringe the lateral margins of pereion somite 1 and epimeral plates 2–4, and are sparse or absent on plates 5–7. Pleotelson elongate, its length is about 1.6 times its width at the base; sides of the pleotelson taper somewhat irregularly from the base to the apex; lateral margins near the apex are very finely denticulate and beset with setae. General body proportions are: body width 0.43 times body length; abdomen length 0.45 times body length; head length about 0.17 times body length.

Antenna 1 is short, extending only to about the end of the peduncle of antenna 2; the flagellum consists of 1 segment and it usually bears 4 pairs of inflated setae on the anterior margin. Antenna 2 is only slightly longer than antenna 1, its flagellum is made up of 3–5 segments. First segment of the peduncle is expanded; second segment with the distal two-thirds expanded; third segment elongate and slender, approximately equal in length to the flagellum. Maxilliped palp is composed of 3 segments. Maxilla 1 with the inner branch possessing a large, plumose seta and a minute seta. Mandible is without a molar process. Propodus of pereiopod 1 is 1.2 times as long as broad; posterior margin of dactyl 1 bears 4–6 fine setae; a few small setae are present on the external lateral surface of the propodus, near the posterior margin. The posterior (ventral) margin of the carpus of pereiopod 1 is armed with only one stout spine. Pereiopods 1, 2, and 3 are generally similar in conformation and embellishment; likewise, pereiopods 4, 5, 6, and 7 resemble one another.

Color.—Basic color of the body and appendages ranges from light tan or a pinkish hue to nearly white with the integument partially covered with dark chromatophores. The chromatophores are black or deep violet and are distributed somewhat unevenly over the body and the larger exposed appendages. Chromatophores are not evident on the pleopods or the inner mouth parts. In the material studied, much variation was observed in the pigmentation pattern from one specimen to another; however, in all specimens examined the chromatophores are consistently more densely concentrated on the uropods and pleotelson than on other parts of the body. Differences in pigmentation appear to be unrelated to size, sex, or season of capture.

Range.—Georges Bank (east of Massachusetts, U. S. A.) in water depths 15 to 36 fathoms and water temperature 45.7° to 57.0° F.

Discussion.—Shape of the head in *C. arenicola* is quite like that found in *C. almyra*. The posterior portion is comparatively long and somewhat narrowed, and the antero-lateral lobes are rather short. The notch separating the antero-lateral margin into two lobes is shallow, as compared to related species. *C. arenicola* is distinguished from all other species in this genus by the absence of setae on the anterior lobe of the antero-lateral margin of the head.

The propodus of pereiopod 1 in *C. arenicola* is relatively short and wide, similar to that of *C. coeca*, but it lacks the setation on the outer central, lateral surface found in that species. The dactyl of pereiopod 1 has only a few thin setae on the occluded margin; in this feature it resembles *C. almyra* and *C. cocca*.

In *C. arenicola* the size and number of setae on the outer margin of epimeral plates 5–7 varies considerably from one specimen to another; however, these setae are generally shorter and much less numerous in this species than in other members of the genus. A slight sexual dimorphism in this feature is apparent

in the few specimens available for study. The setae appear to be longer and more numerous in large males than in adult females and small males.

The shape of the pleotelson of *C. arenicola* is intermediate between that of *C. coeca*, which is broad and tapers irregularly, and that of *C. tuftsi*, which is rather narrow and evenly tapered.

The eye is dark and distinct in some specimens and faint in others. Variation in this characteristic occurs at random among the sexes, specimens of various sizes, and in different collections (some of which have been in preservative for two months and others nearly two years). In the specimens at hand, the eye appears to be proportionately much smaller in *C. arenicola* than that depicted for *C. coeca* by Bousfield (1956).

To my knowledge neither *C. almyra* nor *C. coeca* have been reported from offshore waters, and they have not been observed in our collections of benthic invertebrates from the Georges Bank area. *C. arenicola* and *C. tuftsi* are the only representatives of this genus found in our samples taken during the past few years. Of these two species the latter is far more numerous than *arenicola;* a ratio of approximately 20:1, based on the total number captured. Our records indicate that *C. tuftsi* is rather widely distributed over Georges Bank and nearly always within the 50-fathom isobath. *C. arenicola* seems to be more restricted, having been taken only near the shoals on the north-central part of Georges Bank and on the western end near Great South Channel.

A factor which appears to be important in affecting the distribution of these two species is the particle size composition of the substrate. *C. arenicola* has been taken most frequently from areas where the predominant sediment fraction is a coarse sand (0.5–1.0 mm.). Conversely, *C. tuftsi* is most commonly found where the predominant sediment fraction is a medium sand (0.25–0.5 mm.). Also, there is some evidence that *C. arenicola* either burrows more deeply into the sand or for some other reason is more difficult to evict from the substrate than *C. tuftsi.* This inference is based on two factors: (1) in dredges that normally scrape only the surface of the sea bed, hundreds of *tuftsi* have been caught, compared to only two specimens of *arenicola;* (2) in grab-samplers that usually dig 3 to 6 inches into the sea floor, 10 specimens of *arenicola* have been taken, versus 7 specimens of *tuftsi.*

Some of the more common Crustacea with which *C. arenicola* has been found associated are the amphipods: *Aeginina longicornis* (Kröyer), *Ampelisca spinipes* Boeck, *Amphiporeia virginiana* Shoemaker, *Ericthonius rubricornis* Stimpson, *Haustorius arenarius* (Slabber), *Leptocheirus pinguis* (Stimpson), *Photis dentata* Shoemaker, *Pontogeneia inermis* (Kröyer), *Podoceropsis nitida* (Stimpson), *Sympleustes glaber* (Boeck), and *Tmetonyx nobilis* (Stimpson); the mysid: *Neomysis americana* (Smith); and the decapods: *Cancer borealis*, Stimpson, *Cancer irroratus* Say, *Crangon septemspinosa* Say, *Dichelopandalus leptocerus* (Smith), and *Pagurus acadianus* Benedict. These species are the most abundant crustaceans that were taken in the same bottom-grab samples and dredge hauls with *C. arenicola.* It will be recognized that many of these associates are infauna forms; however, all of the larger species except one are epibenthic. Some species listed above are exceedingly tolerant and are adaptable to diverse environmental features, but others listed here are restricted to very specific types of habitats.

Judging from the information available at this time, it seems likely that *C. arenicola* will be found most closely associated with some of the burrowing amphipods listed above, such as *Amphiporeia, Haustorius,* and *Tmetonyx.*

Key to the Species of Chiridotea[1]

1. Flagellum of antenna *2* much shorter than peduncle, segments 5 or less; antenna 1 nearly as long as antenna *2*. 2

   Flagellum of antenna *2* longer than peduncle, 8–12 segmented; antenna 1 much shorter than antenna *2*. 3

2. Antenna 1 extends beyond peduncle of antenna *2*; margin of anterior lobe of the antero-lateral margin of head is setose. ..... *C. coeca*

   Antenna 1 does not extend beyond peduncle of antenna *2*; margin of anterior lobe of the antero-lateral margin of head is not setose. .... *C. arenicola,* n. sp.

3. Posterior margin of dactyl of pereiopod 1 armed with strong spines; pleotelson narrow, tapering evenly from base to apex. ..... *C. tuftsi*

   Posterior margin of dactyl of pereiopod 1 armed with a few fine setae; pleotelson broad, sides nearly parallel at the basal half. ..... *C. almyra*

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[1] This key modified after that given by Bowman (1955).